Original Article

Histological Study on the Effect of Bone Marrow Derived Mesenchymal Stem Cells Versus their Conditioned Medium and Microvesicles on Experimentally Induced Acute Cerebral Ischemia in Albino Rats

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ABSTRACT

Background and Objectives: Ischemic stroke (an acute cerebrovascular event) is major health problem. It is associated with high rates of mortality and long-term disabilities. The therapeutic options remain limited. This work aimed to evaluate and compare the possible protective effect of bone marrow-derived mesenchymal stem cells (BM-MSCs), their conditioned media (BM-MSCs-CM) and their derived microvesicles (BM-MSCs-MVs) on acute cerebral ischemia.

Materials and Methods: Fifty-two adult male albino rats were divided into five groups: Group I, Group II, Group III, Group IV and Group V. Acute cerebral ischemia was induced in all experimental groups via transient right common carotid artery (RCCA) occlusion. Brain specimens were processed for hematoxylin and eosin, immunohistochemical staining for glial fibrillary acidic protein (GFAP) and vascular endothelial growth factor (VEGF). The mean area percent for both GFAP and VEGF were measured and statically analyzed.

Results: RCCA occlusion resulted in morphological alterations in cerebral cortex of Group II. Significant increase in mean area % of GFAP positive immunoreactivity and significant decrease in mean area % of VEGF positive immunoreactivity was detected in this group as compared to Group I. Instead, the treated groups revealed amelioration in these changes. Significant decrease in mean area % of GFAP positive immunoreactivity along with significant rise in mean area % of VEGF positive immunoreactivity were detected in group V compared to other treated groups.

Conclusion: Administration of BM-MSCs-MVs recorded marked ameliorating effect in comparison to BM-MSCs and BM-MSCs-CM. Hence, it could be considered an ideal candidate as therapeutic agent in ischemic stroke.

Key Words: Conditioned medium, GFAP, Mesenchymal stem cells, Microvesicles.

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INTRODUCTION

Stroke is considered a serious health problem that affects worldwide population. Disability subsequent to stroke imposes heavy burden on both family and society. Stroke incidence is growing with increased age expectancy worldwide. Moreover, modern life stresses and life habits rises stroke incidence among younger people. It is expected to be the main cause of premature death and disability among younger age[1].

Stroke is a cerebrovascular accident that occurs upon interruption of blood flow to brain regions. Strokes are generally classified into ischemic and haemorrhagic. Acute ischemic stroke (AIS) is the most common type. It is caused by occlusion of the supplying artery, triggered by either thrombosis or embolism[2-3-4].

Treatment options for ischemic stroke are still very limited and depend mainly on supportive therapies. Endovascular interventions (including mechanical thrombectomy) and thrombolysis (including intravenous tissue plasminogen activator) are considered the only approved remedies for ischemic stroke[5-6].

These therapeutic options have major limitations due to their narrow time window. Thrombolytic therapy is related to triggering intracranial haemorrhage. Also, thrombectomy is associated with poor outcomes, which are attributed to cerebral ischemic-reperfusion injury[5-6-7].

Inflammatory response and ischemic-reperfusion injury have a fundamental role in ischemic stroke pathophysiology during the acute phase. Additionally, stroke patients are often elderly and suffering from chronic illnesses, which might diminish tissue regenerative potential post stroke[8].

Stem cell-based therapies have been emerged as a promising alternative treatment candidate for ischemic stroke. Bone marrow derived mesenchymal stem cells

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(BM-MSCs) are multipotent cells which are capable of producing several types of specialized cells such as nerve cells and endothelial cells. BM-MSCs administration is considered strong therapeutic option among numerous types of stem cells[9-10-11].

Conditioned medium is harvested from cultured cells. It is comprised of growth factors, metabolites and proteins secreted by the cultured cells. EVs (Extracellular vesicles) are also secreted by stem cells. According to their size, they are classified into microvesicles and exosomes. Microvesicles contains several components e.g. microRNAs which function in post-transcriptional gene regulation. MSC-conditioned medium and EVs including microvesicles are considered alternative therapeutic candidates.[12-13-14-15].

AIM OF THE WORK

The present study was designed to evaluate and compare the possible protective effects of bone marrow-derived mesenchymal stem cells, their conditioned media and their derived microvesicles on experimentally induced acute cerebral ischemia. This was monitored by histological, immunohistochemical and morphometric methods.

MATERIALS AND METHODS

Drugs

Bone marrow derived-mesenchymal stem cells (BM-MSCs), Bone marrow derived stem cells-conditioned medium (BM-MSCs-CM) and Bone marrow derived stem cells-microvesicles (BM-MSCs-MVs): They were prepared and purchased from Biochemistry Department, Kasr Al-Ainy Medical School. PKH26 labelled BM-MSCs were prepared in accordance with **Alhadlaq and Mao (2004)**[16] method. BM-MSCs-CM were prepared according to the method of **Wang et al. (2018)**[17]. PKH26 labelled BM-MSCs-MVs were prepared according to the method of previous studies[18-19].

-Animals and experimental design

This study was conducted at the Animal House of Kasr Al-Ainy Medical School, according to the ethical guidelines for the care and use of laboratory animals. Fifty-two male albino rats 3 months old (150-200 grams) were used in this experiment. They were housed in hygienic stainless-steel cages. They were fed standard chow diet and allowed free access to water.

The rats were allocated into 5 groups:

Group I (control group) (n; 12): Rats were subdivided into:

• **Subgroup Ia (n; 6)**: apparently normal animals which did not subjected to any surgical intervention. They received only the vehicle (single intravenous injection of 1ml normal saline).

• **Subgroup Ib (Sham-Operated group) (n; 6):** subjected to sham operation, then they received the vehicle.

Animals of both subgroups were sacrificed with groups II, III, IV and V.

Experimental groups (n; 40):

Ischemia-reperfusion injury (IRI) was induced in all experimental groups (as discussed below), then they were subjected to the following:

• **Group II (untreated ischemic group) (n; 10)**: They were given intravenous 1ml normal saline injection, immediately after reperfusion, once via tail vein.

• **Group III (BM-MSCs treated group) (n; 10)**: Immediately after reperfusion, they received single intravenous injection of BM-MSCs via tail vein. PKH26 labelled BM-MSCs were in a dose of 1x106, diluted in 1ml of saline, loaded in a 1ml sterile syringe[6-20].

• **Group IV (BM-MSCs-CM treated group) (n; 10)**: They received single intravenous injection of BM-MSCs-CM immediately after reperfusion. BM-MSCs-CM was given in a dose of 100 ml, diluted in 1 ml of saline, loaded in a 1ml sterile syringe[21-22].

• **Group V (BM-MSCs-MVs treated group) (n; 10)**: They received single intravenous injection of BM-MSCs-MVs immediately after reperfusion. BM-MSCs-MVs was given in a dose of 100 ml, diluted in 1ml of saline, loaded in a 1ml sterile syringe[23-24].

Rats of all groups were sacrificed 24h after injection.

-Induction of cerebral ischemic injury by occlusion of right common carotid artery:

This was achieved according to a method modified from **Mentari et al. (2018)**[25]. Animals were anaesthetized using mixture of ketamine and Xylazine (80 mg/kg and10 mg/kg, respectively). Right common carotid artery (RCCA) was exposed via small midline incision in the neck. Then it was isolated and separated from the vagal nerve and surrounding connective tissue by blunt dissection. RCCA was ligated using silk suture for 60 minutes. After 60 minutes the ligature was removed. Immediately after reperfusion all experimental groups received single intravenous injection of either saline or BM-MSCs or BM-MSCs-CM or BM-MSCs-MVs. For sham operated group, the same steps were done except for RCCA occlusion. Post operatively lidocaine gel was applied to relieve wound pain.

-Histological examination: At the end of the experimental period each rat received intraperitoneal

injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Chest wall was opened, then the descending aorta was ligated. Next, the left ventricle was perfused with 10% formalin. Instantly after perfusion, a small opening was made in right atrium. Perfusion was stopped as soon as the venous return from the right atrium turned into clear[26].

Then, brain was dissected and fixed in formalin (10%), dehydrated, cleared then embedded in paraffin wax. Paraffin blocks were cut at thickness of 5µm. Then mounted on glass slides and subjected to the following:

1) **Hematoxylin & Eosin (H&E) stain**[27].

2) **Immunohistochemical staining: using the following primary antibodies**[28]:

a- Glial fibrillary acidic protein (GFAP) for visualization of reactive and damaged astrocytes.

b- Vascular endothelial growth factor (VEGF) which is a marker for angiogenesis and vascular changes.

Immunohistochemical staining were done using the technique of avidin biotin peroxidase complex for GFAB (a mouse monoclonal antibody, PA5-16291, Lab Vision Corporation laboratories) and for VEGF (a rabbit polyclonal antibody, RB-222-R7, Lab Vision Corporation Laboratories). The detection system histostain SP kit was used (LAB-SA system, Zymed Laboratories Inc, SF, USA). Counter staining of nuclei was done using Mayer's haematoxylin stain. GFAB positive immune reaction appeared as brown cytoplasmic deposits. VEGF positive immune reaction appeared as brown nuclear and cytoplasmic deposits[29].

3) Unstained sections were examined to detect PKH26 labelled BM-MSCs and BM-MSCs-MVs, using **fluorescent microscopy** (Olympus BX50F4, No. 7M03285, Tokyo, Japan).

Morphometric studies:

Morphometric study was conducted to measure the mean area percent of both GFAP immune expression and VEGF immune reactivity.

Data was obtained using Leica Qwin 500 C LTD image analyzer computer system (Cambridge, UK), at Histology Department, Faculty of Medicine, Cairo University. The measurements were done in 10 random non overlapping fields per section, using the binary mode, at magnification of x400.

Statistical analysis:

Measurements were expressed as means ± standard deviation and were analyzed statistically using the SPSS software (version 16). This was done using one-way analysis of variance followed by Tukey's post-hoc test. Results were considered significant when the *P value* was < 0.05[30].

RESULTS

No incidence of mortality was observed in rats during the experimental period. Histological and morphometric results of both subgroups Ia and Ib expressed no differenes. Thus, they were referred to by group I (control group).

Histological Results:

(I) Hematoxylin and Eosin Results

Examination of right cerebral cortex sections of control group revealed its normal histological architecture. Cerebral cortex demonstrated variable types and shapes of neurons which cannot be easily differentiated from one another in H&E sections. Together with neurons, glial cells were dispersed in-between. Neurons were distinguished from glial cells by their large spherical, pale stained nuclei with prominent nucleoli and basophilic cytoplasm. Their processes were barely visualized. Multiple glial cells appeared as small cells with dark nuclei scattered in-between neurons. Axonal, dendritic and glial processes were tangled into eosinophilic fibrillar material, called neuropil, in-between neurons and glial cells. Meninges covering cerebral cortex could also be detected (Fig. 1A).

Cerebral cortex of untreated ischemic group showed multiple deformed and shrunken neurons. Other neurons exhibiting either darkly stained pyknotic nucleus or dissoluted nuclei were also demonstrated. In addition, neuron with dark fragmented nucleus and acidophilic cytoplasm was detected with halo surrounding it. Blood vessels represented endothelial lining with dark nuclei and were surrounded by wide perivascular spaces denoting edema. Vacuolations within the neuropil were also visualized (Figs. 1B,1C&1D).

BM-MSCs, BM-MSCs-CM and BM-MSCs-MVs treated groups right cerebral cortex demonstrated few deformed and shrunken neurons. Many normal neurons were observed. Congested blood vessel surrounded by mild perivascular space/edema was detected in BM-MSCs treated group. A Blood vessel demonstrating minimal perivascular space/edema was still evident in BM-MSCs-CM treated group. Normal blood vessels were obvious in both BM-MSCs and BM-MSCs-MVs treated groups (Figs. 2A,2B&2C).

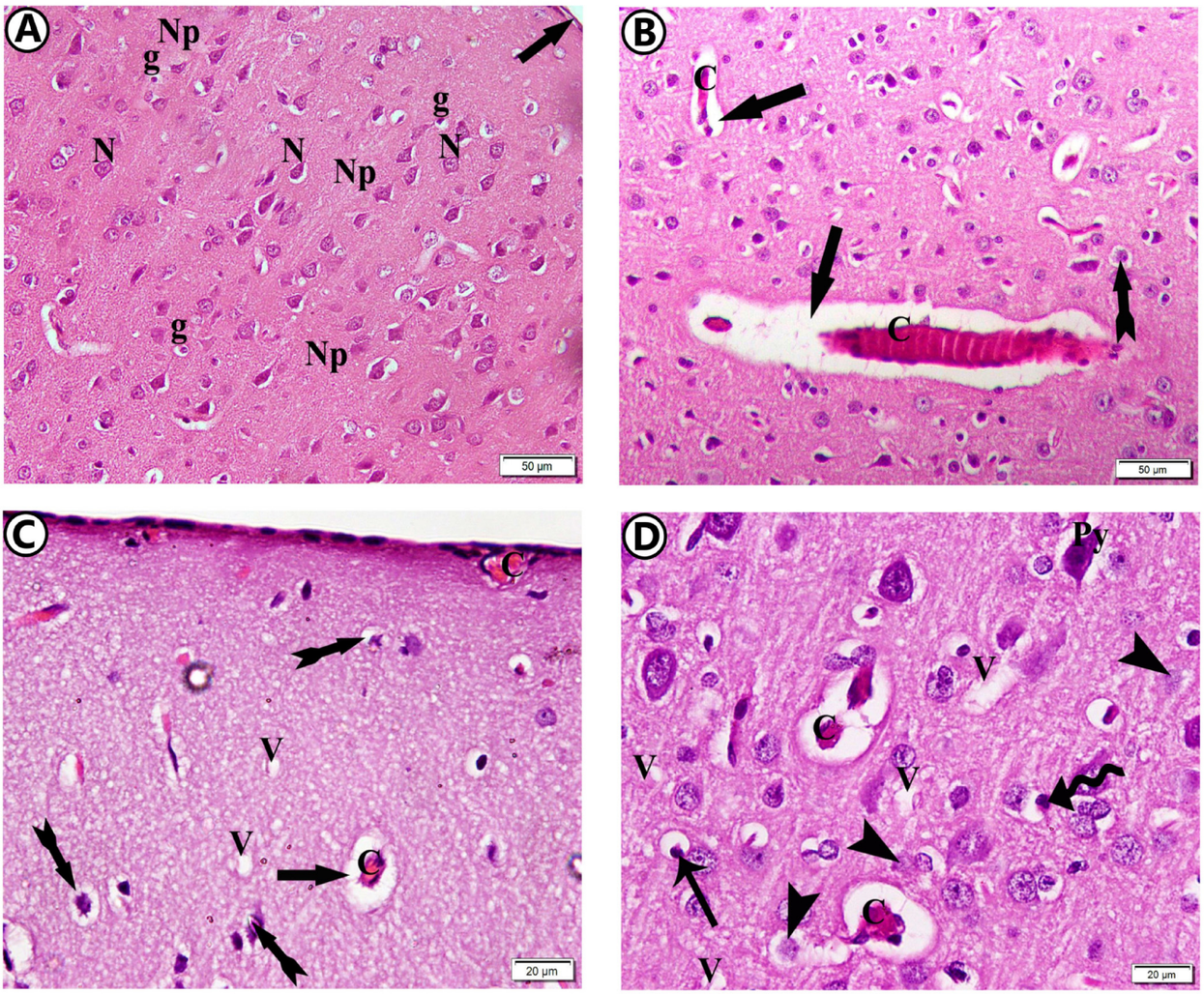

Fig. 1: Right cerebral cortex of: (A) Group I (control) showing normal histological architecture of cerebral cortex. It is composed of variable types of neurons and glial cells. Neurons (N) can be distinguishable from glial cells by having large spherical, pale stained nuclei with prominent nucleoli and basophilic cytoplasm. Their processes are barely visualized. Multiple glial cells (g) appear as small cells with dark nuclei scattered in-between neurons. An eosinophilic meshwork formed of axonal, dendritic and glial processes could be visualized and it is called neuropil (Np). Meninges covering cerebral cortex can also be detected (black arrow). (B, C& D) Group II demonstrating deformed and shrunken neurons surrounded with a halo (bifid arrows). Neurons exhibiting either darkly stained pyknotic nucleus (Py) or dissoluted nucleus (arrowheads) can be also detected. Neuron with dark fragmented nucleus with acidophilic cytoplasm and surrounded by halo (wavy arrow) is demonstrated. Neuron with shrunken dark nucleus with acidophilic cytoplasm and surrounded by a halo is also seen (thin arrow). Blood vessels exhibiting endothelial lining with dark nuclei and surrounded by wide perivascular space can be detected (arrows). Vacuoles (V) within the neuropil are another finding. (A&B; H&E, x200, C&D; H&E, x400***).***

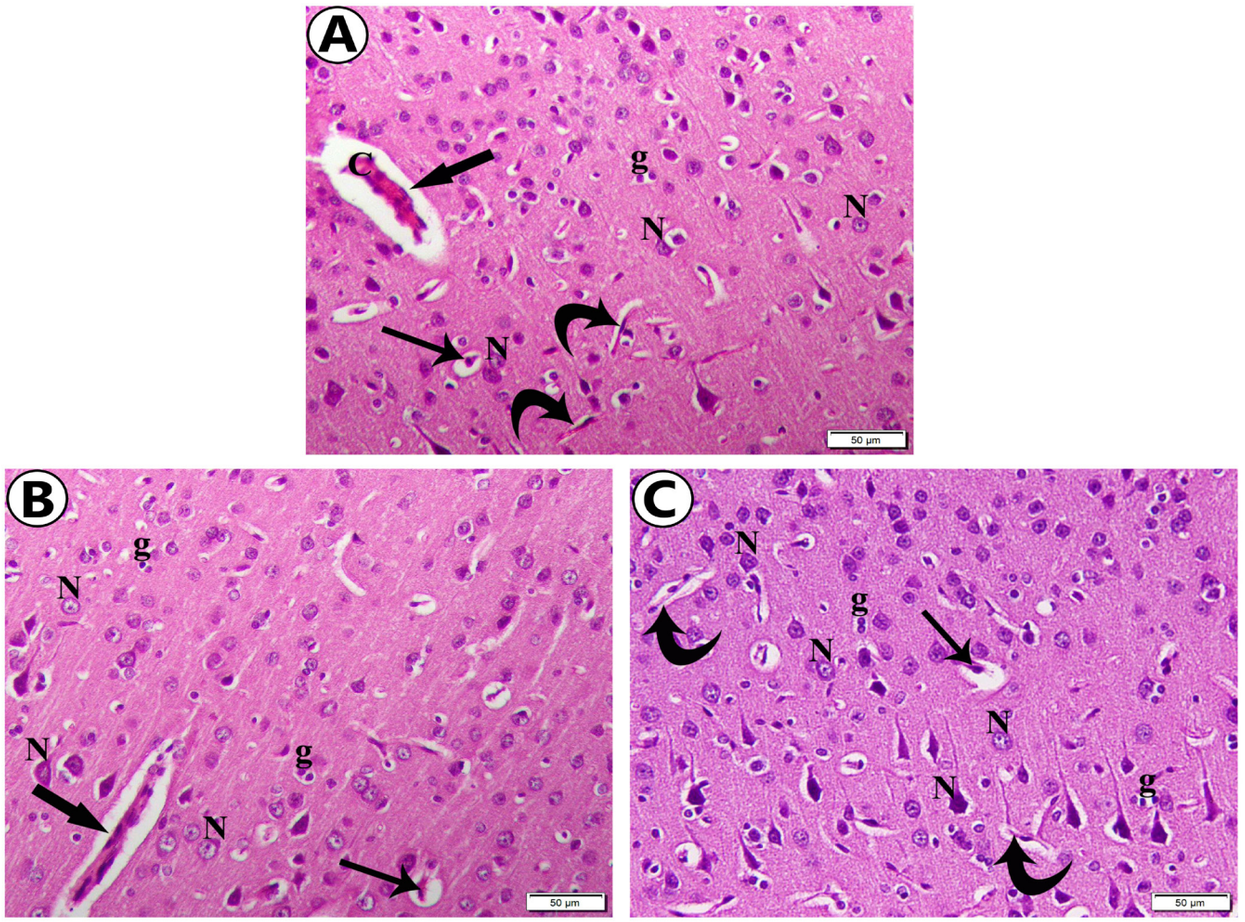

Fig. 2: A, B and C represents sections in the right cerebral cortex of treated groups (groups III, IV & V, respectively) showing preserved normal histological structure with multiple normal neurons. Few deformed and shrunken neurons (thin arrows) exhibiting shrunken dark nucleus with acidophilic cytoplasm and surrounded by a halo can be detected. Moreover, congested blood vessel surrounded by mild perivascular space (arrow) can be detected in group III. A blood vessel surrounded by minimal perivascular space (arrow) is also seen in group IV. Normal blood vessels (curved arrows) are demonstrated in both groups III and V sections. (A, B & C; H&E, x200)..

(II) Immunohistochemichal Results:

GFAP Immuno- Stained Sections:

Ipsilateral cerebral cortex sections of group I exhibited scanty brown positive GFAP immunoreactivity in the cytoplasm of protoplasmic astrocytes and their processes (Fig. 3A). Group II showed marked increase in GFAP positive immunoreaction in the cytoplasm of the reactive astrocytes and their processes (Fig. 3B). Group III demonstrated moderate rise in positive cytoplasmic GFAP immunoreactivity of reactive astrocytes and their processes (Fig. 3C). Group IV showed mild increase in GFAB positive immunoreactivity of reactive astrocytes and their processes (Fig. 3D). The least increase in GFAB positive immune reaction was detected in astrocytes and their processes of group V (Fig. 3E).

VEGF Immuno-Stained Sections:

Right cerebral cortex sections of control group showed weak positive VEGF immunoreactivity within neurons, glial cells and endothelial cells. (Fig. 4A). Group II exhibited mild increase in VEGF immuno-positive reaction in neurons and glial cells (Fig. 4B). Group III showed moderate rise in VEGF positive immunoreaction in neurons and glial cells (Fig. 4C). Group IV showed that neurons, glial cells and endothelial cells of blood vessels demonstrated marked increase in VEGF positive immunoreaction (Fig. 4D). The most abundant increase in VEGF positive immunoreactivity was detected in group V (Fig. 4E).

(III) Fluorescent Microscopy Results:

BM-MSCs and BM-MSCs-MVs appeared as red fluorescent dots, confirming their seeding into brain tissue. Sections in cerebral cortex of both groups III and V showed PKH26 labelled MSCs and MVs, respectively (Fig. 5).

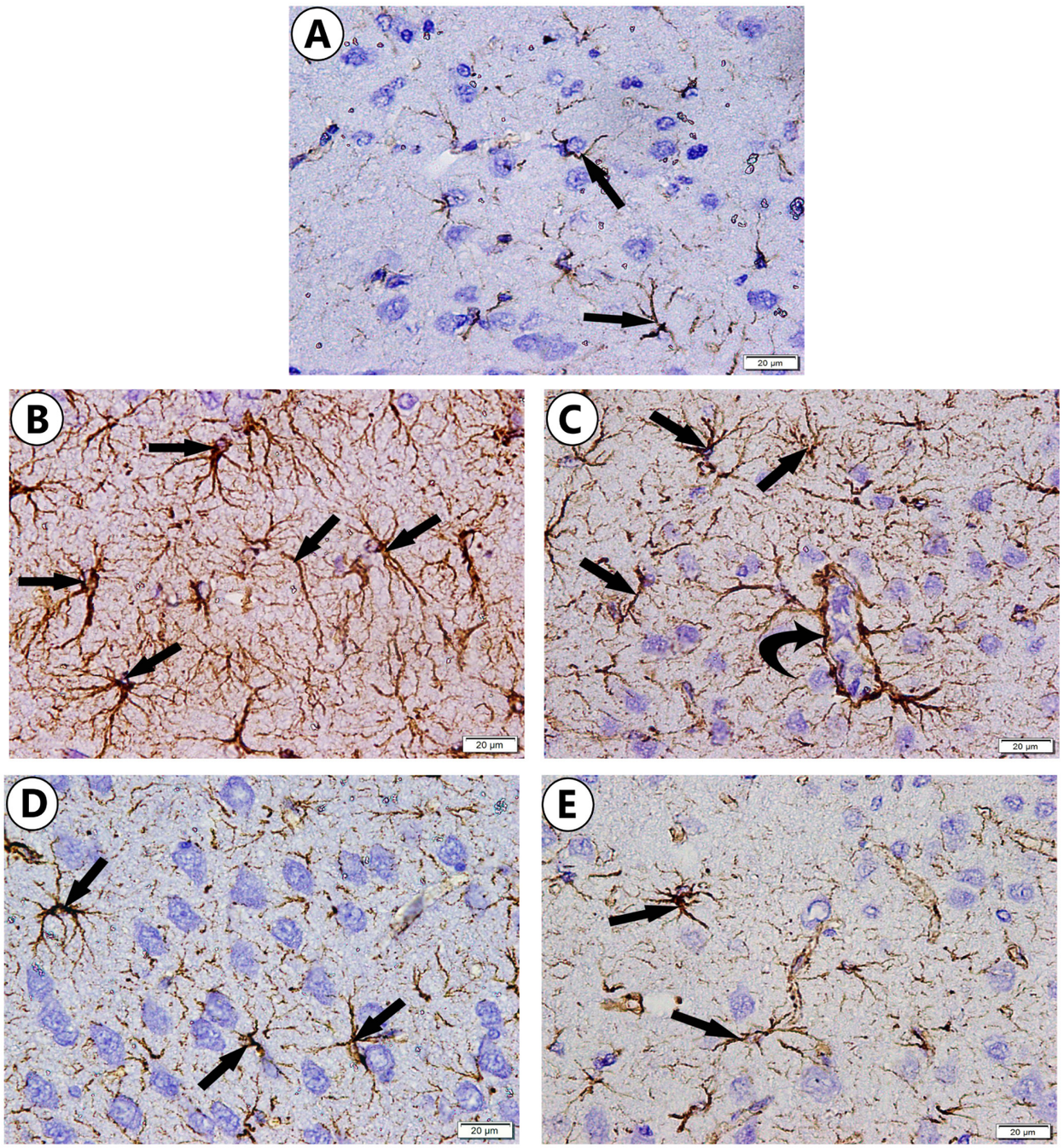

Fig. 3: Right cerebral cortex of: (A) Control group showing scanty positive brown cytoplasmic immunoreaction for GFAP within protoplasmic astrocytes and their processes (black arrows). (B) Group II expressing marked increase in GFAB positive immunoreactivity within the cytoplasm of reactive astrocytes and their processes (black arrows). (C) Group III exhibiting moderate increase in GFAP positive immune reaction within the reactive astrocytes and their processes (black arrows). Note the blood vessel which is surrounded by astrocyte feet (curved arrow). (D) Group IV showing mild rise of reactive astrocytes and their processes GFAP positive immunoreaction (black arrows). (E) Group V revealing weak positive immunoreaction for GFAP in reactive astrocytes and their processes (black arrows). (A, B, C, D &E; GFAP Immunohistochemistry, x400).

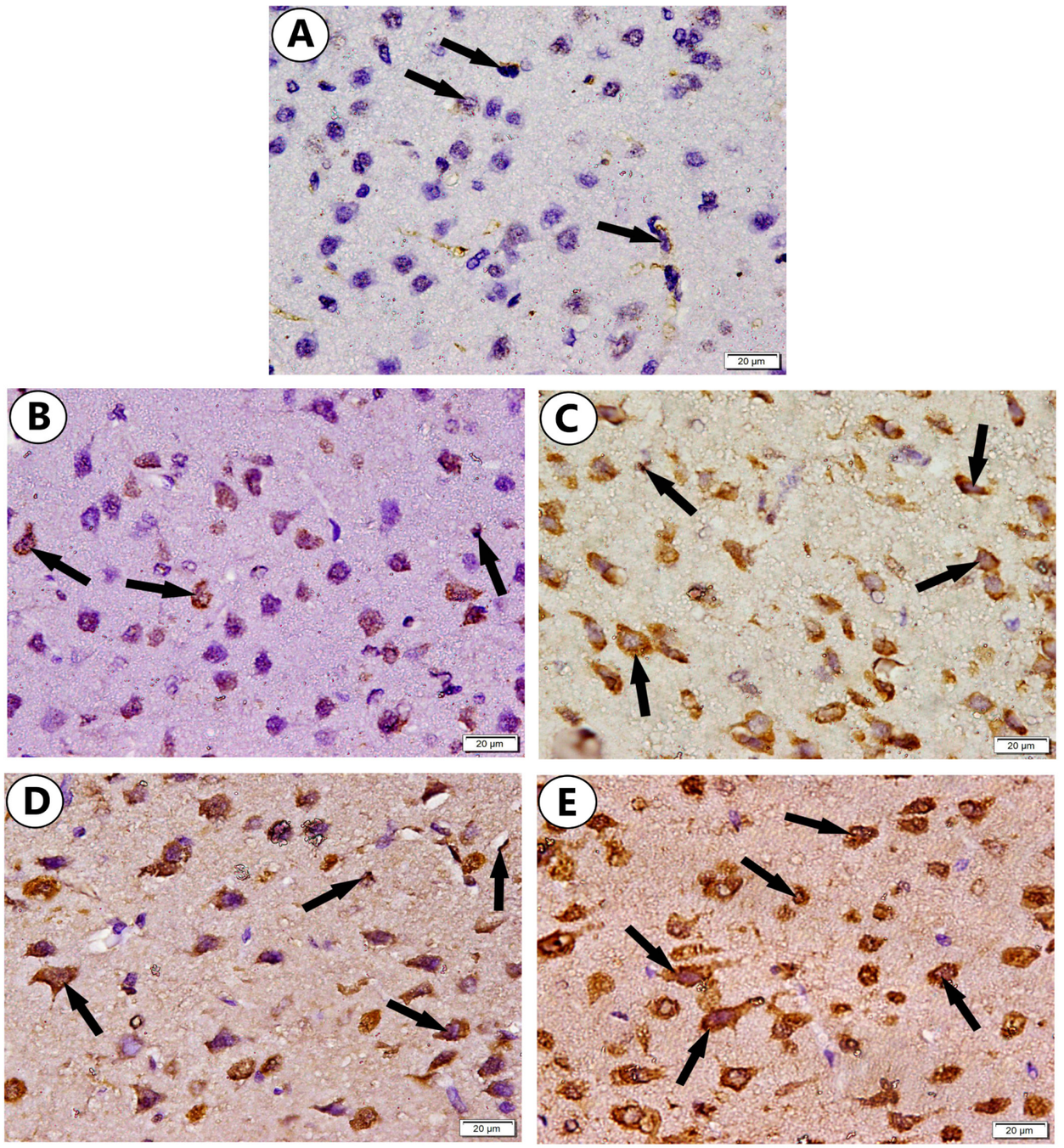

Fig. 4: Right cerebral cortex of; (A) Control group demonstrating weak positive immunoreaction for VEGF (black arrow). (B) Untreated ischemic group showing mild positive immuno-expression for VEGF (black arrows). (C) BM-MSCs treated group showing moderate increase in VEGF immuno-positive reaction (black arrows). (D) BM-MSCs-CM treated group showing marked increase in immuno-positive reaction of VEGF (black arrows). (E) BM-MSCs-MVs treated group revealing strong positive immuno-expression for VEGF (black arrows). (A, B, C, D &E; VEGF Immunohistochemistry, x400***).***

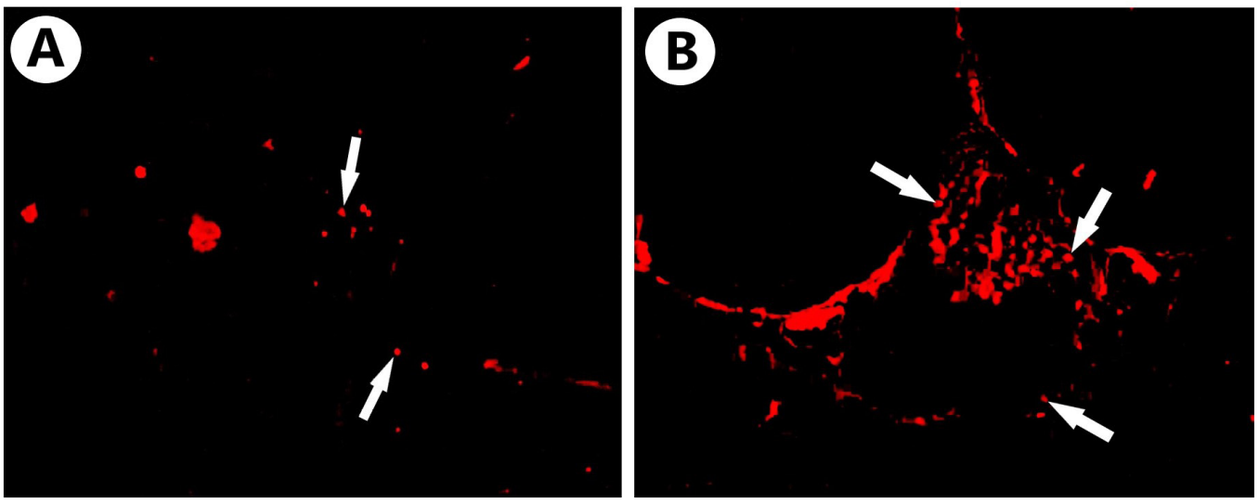

Fig. 5: Cerebral cortex of; (A) Group III expressing BM-MSCs labelled with PKH26 (arrows). (B) Group V showing PKH26 BM-MSCs-MVs (arrows). (A&B; PKH26 Fluorescent dye, x400).

Morphometric Results:

Values of the mean area percent of GFAP positive immunoreactivity of groups II, III and IV revealed a significant increase when compared to control. Group III and IV values reported significant decrease in comparison to group II. Also, group IV revealed significant decline compared to group III. No significant difference reported between group V and control. Additionally, significant decrease was revealed in group V in relation to groups II, III and IV.

Regarding the mean area percent of VEGF positive immunoreactivity, groups II, III, IV and V revealed statistically significant increase when compared to control. All experimental groups exhibited significant rise in relation to group II. Significant increase in group V when compared to all experimental groups was also detected.

Table: Mean values of area percent of GFAP and VEGF immunoreaction (±SD) in the studied groups.

Groups	The mean area percent (±SD) of GFAP immunoreaction	The mean area percent (±SD) of VEGF immunoreaction
Group I	0.27 ± 0.09	0.79 ± 0.40
Group II	5.25 ± 1.99*	4.88 ± 0.49*
Group III	4.19 ± 1.02*•	12.84 ± 1.63*•
Group IV	1.68 ± 0.44*•⌂	20.74 ± 1.19*•
Group V	0.46 ± 0.26•⌂#	29.87 ± 1.83*•⌂#

* Significantly different as compared to the control group ($P < 0.05$).
• Significantly different as compared to group II ($P < 0.05$).
⌂ Significantly different as compared to group III ($P < 0.05$).
Significantly different as compared to group IV ($P < 0.05$).

DISCUSSION

Acute ischemic stroke (AIS) is one of the principal causes that lead to long-term disability and death. Tissue plasminogen activator (tPA) and/or mechanical thrombectomy are the most effective therapies for AIS, facilitating reperfusion of the occluded causative vessel[31-32-33].

Nevertheless, this treatment could be only applied to 5% or less of ischemic stroke patients, due to the narrow time window. Hence, novel treatment strategies with less complications are required[34-35].

The present experimental study was conducted in order to test the protective benefits of BM-MSCs, their conditioned medium (BM-MSCs-CM) and their microvesicles (BM-MSCs-MV) in a rat model of cerebral ischemia. This was monitored by histological, immunohistochemical and morphometric studies.

In this study, we employed cerebral ischemia model to study the histological changes that occur post-stroke. Transient unilateral right common carotid artery occlusion was used to induce ischemic injury in the ipsilateral cerebral hemisphere.

Common carotid artery blockage is commonly associated with internal carotid artery (ICA) occlusion with subsequent ischemia in the brain territory supplied by the ICA[36-37-38]. Similarly, **Das** *et al*. **(2018)**[39] reported that transient focal ischemia was induced via unilateral common carotid artery occlusion.

The light microscopic examination of H&E-stained sections of the ipsilateral cerebral cortex of the untreated ischemic group revealed marked histological changes.

Multiple deformed and shrunken neurons surrounded with a halo, neurons exhibiting pyknotic nucleus or dissoluted nucleus and others with fragmented dark nucleus, acidophilic cytoplasm and surrounded with a halo were demonstrated. In addition, the presence of blood vessels exhibiting endothelial lining with dark nuclei was seen. Congested blood vessels with wide perivascular spaces denoting edema were detected. Moreover, vacuoles within neuropil were also evident.

In line with these findings, previous researchers[40,41] and[42] documented that neurons retracted from the surrounding neuropil were detected in early stage of ischemic damage. Moreover, areas of ischemic damage revealed vacuolations and sparseness (spaces) of the neuropil. Besides, **Mărgăritescu** *et al.* **(2009)**[43] demonstrated the earliest signs of neuronal damage after acute ischemic brain insults. Neurons with shrunken darkly stained nucleus and eosinophilic cytoplasm were reported. As well, Fading and dissolution of nuclei and fragmented nuclei are considered other features of cellular degeneration[44].

The deformed and shrunken neurons can be explained by previous work[45] who documented that obstruction of blood flow to brain leads to loss of oxygen and glucose supply. This anaerobic state leads to mitochondrial dysfunction, calcium dysregulation, endoplasmic reticulum stress and apoptosis.

Furthermore, the explanation for the deformed neurons and edema can be obtained from multiple studies of[46,47,48] and[49] reported that stroke leads to series of complex events including oxidative stress and disruption of the blood-brain barrier (BBB) leading to edema and inflammation that result in cell death. Increased vascular permeability also permits entry of substances and immune cells which are prohibited in normal conditions by BBB causing neuroinflammation. Thus, ischemia immediately results in oxidative stress, inflammatory reaction and BBB disruption, which altogether leads to death of neurons, glial cells and endothelia cells.

The presence of dark endothelial cells can be supported by **Hollande** *et al.* **(2016)**[50] who reported that endothelial nuclei exhibited signs of early apoptosis in ischemia with DNA condensation. As regard the observed perivascular edema, edema around blood vessels in acute ischemic brain insults was previously noted[51]. The congestion in blood vessels in the brain was similarly observed in cases of ischemic stroke[52].

The presence of perivascular edema and vacuolations within neuropil (spongiosis) in ischemic stroke were attributed to alterations in brain microvasculature and endothelial dysfunction[43-53]. This results in plasma extravasation through blood vessels wall and leakage into brain tissue in area of ischemic insult causing perivascular edema and spongiosis. It was stated[54] that a compensatory cerebral autoregulation to keep constant cerebral blood flow result in maximal dilation of blood vessels and congestion.

In the present study, immunohistochemical staining was used to evaluate GFAP expression. Glial fibrillary acidic protein (GFAP) is the intermediate filament protein in astrocytes which are the main type of glial cells in the brain. GFAP levels are considered complementary biomarker to predict functional outcome following AIS[55-56-57].

Morphometric measurements of the mean are percent of GFAP positive immunoreactivity for untreated ischemic group showed statistically significant increase when compared to control. In accordance with our findings, other works[58] and[59] demonstrated widespread reactive astrogliosis with increased levels of GFAP expression in the cortex just one day after ischemic insult. It was also recorded[60] that hypoxia depolarize astrocytes to be reactive.

Stroke induces astrocytes proliferation and increase levels of GFAP in a process known as reactive astrogliosis. Reactive astrogliosis is considered a key pathological hallmark of altered brain tissue. The glial scar is the most prominent feature of this process and it represents maladaptive phenomenon which causes inflammation, neurotoxicity and axon regeneration inhibition[61]. So, it is noteworthy to focus on decreasing astrogliosis to provide proper environment for neurogenesis[13].

In the present study, immunohistochemical staining with VEGF was used to detect its expression in cerebral cortex after injury. Morphometric measurements showed statistically significant increase in mean area percent of VEGF positive immunoreactivity of untreated ischemic group as compared to control. This finding correlated with a study[62], who demonstrated that the percentage of VEGF positive immunoreactivity was significantly higher in ischemic rat brain compared to control at two hours after ischemic insult and was sustained for two weeks later. It was[63] reported that VEGF expression was demonstrated in astrocytes, neurons and endothelial cells. Vascular endothelial growth factor (VEGF) is considered a key mediator of angiogenesis. Also, there is evidence of its role in neurogenesis and neuroprotection providing fast functional recovery after injury. During cerebral ischemia, VEGF affects neural development and neovascularization in brain[64].

VEGF is the most effective hypoxia triggered angiogenic factor which is released by endothelial cells. It possesses strong effect on new vessels growth in the injured area of brain[65]. Also, it can promote angiogenesis

and neurogenesis together with neuroprotection. It was reported that VEGF induces astrocyte trans differentiation into new neurons after acute ischemic insult, hence encouraging neurogenesis[66].

In the current study, the morphological alterations detected in the treated groups (BM-MSCs, BM-MSCs-CM and BM-MSCs-MVs) were attenuated compared to the untreated ischemic group. Also, the findings showed that BM-MSCs-CM administration attenuated post ischemic changes to extent that was greater than BM-MSCs. Moreover, examination of cerebral cortex sections obtained from BM-MSCs-MVs treated group revealed that MVs resulted in the greatest protective effect in injured rats.

In agreement with the previously mentioned findings, reduction in the damaged tissue after MSCs therapy was observed when compared to the untreated ischemic group[67]. Likewise, an experimental study[68] was performed to detect the effects of BM-MSCs-CM on ischemic stroke animal model. The results proved that the BM-MSCs-CM declined brain damage after ischemic stroke and it was associated with few deformed neurons. Additionally, MSCs-MVs were reported to be effective in ameliorating ischemic brain injury and in enhancing functional recovery[13].

As regards GFAP immune expression, morphometric measurements of the mean are percent of GFAP positive immunoreactivity exhibited significant decline in all treated groups in comparison to untreated ischemic group. BM-MSCs-MVs treated group recorded the most decreased values which were comparable to that of control. Statistically significant decrease was reported in group V in relation to both groups III and IV. Furthermore, group IV demonstrated statistically significant decrease relative to group III.

The previous data went parallel with a study[69] which demonstrated that administration of MSCs and MSC-CM reduced GFAP expression. Similarly, it was detected that EVs administration significantly reduced density of GFAP positive astrocytes in injured cortex[70].

Regarding VEGF immunohistochemical expression, the most abundant VEGF positive immunoreactivity was recorded in BM-MSCs-MVs treated group. Morphometric measurements confirmed that BM-MSCs, BM-MSCs-CM and BM-MSCs-MVs treated groups expressed statistically significant increase when compared to untreated ischemic group. Moreover, BM-MSCs-MVs treated group exhibited significant rise in comparison to both BM-MSCs and BM-MSCs-CM treated groups.

In accordance with these findings, it was shown[71] and[72] that BM-MSCs augmented VEGF expression. Besides, MSCs-CM contains cytokines which upregulates VEGF synthesis[73]. Also, it was[74] documented that BM-MSCs derived vesicles increased post-ischemic angiogenesis and this was detected by rise in VEGF expression.

The ability of BM-MSCs, BM-MSCs-CM and BM-MSCs-MVs to ameliorate ischemic changes after acute cerebral ischemic insult could be explained by previous studies. It was[75], reported that the neuroprotective effects of MSCs are attributed to its ability to dampen cell death responses associated to stroke. This is endorsed to anti-oxidative stress and anti-inflammatory capability associated with reducing mitochondrial impairment, and apoptosis. Moreover, it was[76] stated that transplanted MSCs could secrete various growth factors and cytokines. These factors could augment the process of neurogenesis and angiogenesis.

Furthermore, it was[77], postulated that MSC-CM could promote tissue repair through several mechanisms. These mechanisms include cell death prevention and modulation of inflammatory process. Moreover, neurogenesis and angiogenesis are another suggested mechanism. Also, it was[78], documented that MSC-CM can reduce BBB leakage and enhance astrocyte survival associated with GFAP downregulation.

Besides, EVs including MVs are considered a good alternative for MSC application. Experimental studies revealed that EVs have the same effects as the cells from which they were derived[79]. Moreover, they have extra advantages in terms of low immunogenicity and no risk of vessel obstruction. Furthermore, intravenous injection of MSC-derived microvesicles could enhance angiogenesis and neurogenesis improving functional recovery,[80] and[81] showed that. Similar suggestions were[82,83,84] and[85] postulated that microRNA (small non-coding RNA molecules) content enclosed within the vesicles regulates gene expression. Several mechanisms were proposed to elucidate the protective effects of miRNA content. These include anti-oxidative stress, anti-inflammatory, anti-apoptotic effects in addition to protection of BBB and neurogenesis. Angiogenesis is also controlled by various miRNAs through upregulation of VEGF levels.

In the light of the previous findings and data, it could be elucidated that intravenous administration of BM-MSCs, BM-MSCs-CM and BM-MSCs-MVs played a crucial role in neuroprotection after induced acute ischemic cerebral damage. Fulfilling the aim of the current study, comparing the results of all treated groups clarified that BM-MSCs-MVs administration could be considered the best of the used agents.

CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربى

دراسة هستولوجيه على تأثير الخلايا الجذعية الوسطية المشتقة من نخاع العظم مقابل الوسط المكيف والحويصلات الدقيقة لها على نقص التروية الدماغي الحاد المستحث تجريبياً في الجرذان البيضاء

صافيناز صلاح الدين سيد ـ سامح السنباطى ـ غاده محمد ابراهيم و منال على عبدالمحسن

الخلفية و الأهداف: تعتبر السكتة الدماغية (وهي حدث وعائي دماغي حاد) مشكلة صحية كبيرة. ترتبط بارتفاع معدلات الوفيات والإعاقات طويلة الأمد. و لا تزال الخيارات العلاجية محدودة. يهدف هذا العمل إلى تقييم ومقارنة التأثير الوقائي المحتمل للخلايا الجذعية الوسطية المشتقة من نخاع العظم (BM-MSCs) والوسط المكيف لها (BM-MSCs-CM) والحويصلات الدقيقه المشتقة منها (BM-MSCs-MVs) على إقفارالدماغ الحاد.

المواد والطرق: تم تقسيم إثنان وخمسون فأرًا بالغًا من ذكور الجرذان البيضاء إلى خمس مجموعات: المجموعة الأولى ، المجموعة الثانيه ، المجموعة الثالثه ، المجموعة الرابعة و المجموعة الخامسة. تم إحداث نقص التروية الدماغي الحاد في جميع المجموعات التجريبية عن طريق انسداد الشريان السباتي المشترك الأيمن (RCCA). تمت معالجة عينات الدماغ بصبغة الهيماتوكسيلين والإيوسين ، وصبغه كيميائيه مناعيه للبروتين الحمضي الليفي الدبقي (GFAP) وعامل نمو بطانة الأوعيه الدمويه (VEGF). تم قياس متوسط المساحة المئويه لكل من GFAP و VEGF وتحليلهما بشكل إحصائى.

النتائج: أدى انسداد RCCA إلى تغيرات شكلية في القشرة الدماغية للمجموعة الثانيه. تم الكشف عن زيادة ملحوظه في متوسط المساحة المئويه من النشاط المناعي الإيجابي لـ GFAP وإنخفاض ملحوظ في متوسط المساحة المئويه من النشاط المناعي الإيجابي لـ VEGF في هذه المجموعة بالمقارنة مع المجموعه الضابطه. عوضا عن ذلك ، كشفت المجموعات المعالجة (الثالثه ، الرابعه ، الخامسه) عن تحسن في هذه التغييرات. تم الكشف عن إنخفاض ملحوظ في متوسط المساحة المئويه من النشاط المناعي الإيجابي لـ GFAP مصاحب بإرتفاع ملحوظ في متوسط المساحة المئويه للنشاط المناعي الإيجابي لـ VEGF في المجموعة الخامسة مقارنة بالمجموعات المعالجه الأخرى.

الخلاصه: سجلتBM-MSCs-MV تحسن ملحوظ بالمقارنة مع BM-MSCs و BM-MSCs-CM. وبالتالي ، يمكن اعتبارها مرشحًا مثاليًا كعامل علاجي في السكتة الدماغية.