

Combined Treatment of Levetiracetam and Mesenchymal Stem Cells Reverses the Biochemical Aberrations in the Acute Phase of Epilepsy Induced by Pilocarpine in Rats

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In spite of the enormous evolution of the novel anti-seizure medications, about one-third of epilepsy patients stay resistant to the existing therapeutic drugs. Stem cells have provoked hopeful for treating diverse neurologic diseases comprises epilepsy. The rational of this investigation was to appraise the therapeutic intervention of a combination of levetiracetam (LEV) with rodent adipose-derived mesenchymal stem cells (ADMSCs) or rodent bone marrow-derived mesenchymal stem cells (BMMSCs) in counteracting pilocarpine-induced acute epilepsy in rats. In this research, the isolation and preparation of ADMSCs and BMMSCs from male albino rats were carried out. The identification of ADMSCs and BMMSCs was performed morphologically in the culture by using the inverted microscope and by the detection of the cell surface profile by using the flow cytometry technique. The induction of acute epilepsy was achieved by intraperitoneal injection of a single dose of pilocarpine (380 mg/kg b.wt). This study was conducted on fifty six adult male albino rats which were assigned into seven equal groups (8 rats/group); Group (1): Control, Group (2): Epileptic, Group (3): Epileptic + LEV (300 mg/kg b.wt daily for 12 weeks by gastric intubation with an oral gavage), Group (4): Epileptic + ADMSCs (single dose of ADMSCs; 3×10^6 cells/rat; intravenously), Group (5): Epileptic + BMMSCs (single dose of BMMSCs 3×10^6 cells/rat; intravenously), Group (6) : Epileptic + ADMSCs + LEV and Group (7) Epileptic + BMMSCs + LEV. After the end of the experimental period (12 weeks), all rats were tested by the mean electric shock (MES) test to prove the presence of strong seizures in the epileptic group and the modulation of these seizures after treatments. Then, the rats were decapitated and the whole brain of each rat was dissected into two halves, the first half was used for the quantitative determination of GABA, glutamate, dopamine, bFGF, BDNF, IL-6 and TNF- α , while the second half was fixed in formalin saline for histological investigation. The findings of the present work demonstrated that the morphological appearance of the isolated MSCs manifests spindle-shape. The flow cytometric analysis showed that the isolated MSCs are positive for CD90 and negative for CD14 and CD45. The homing of MSCs in the brain tissue of the treated rats was verified by their staining with the fluorescent dye. The recordings of the MES indicated the presence of strong seizures in the epileptic rats which were ameliorated after treatment with LEV, ADMSCs, BMMSCs. The level of brain GABA decreased significantly in the epileptic rats, whereas the level of brain glutamate, dopamine, bFGF, BDNF, IL-6 and TNF- α increased significantly; these alterations were improved after treatment with LEV or ADMSCs or BMMSCs. The histological examination of the brain tissue of the epileptic rats showed great histopathological alterations which were amended by the different treatment options. The combined treatment of either ADMSCs or BMMSCs with LEV displayed superior advantageous effect versus the single use of each type of cell in combating

the acute phase of epilepsy. In conclusion, the outcomes of the present approach disclosed that the combined treatment of either ADMSCs or BMMSCs with the antiepileptic drug LEV has synergistic effect in alleviation of the behavioral and biochemical aberrations as well as brain histological deformation during the acute phase of epilepsy.

Keywords: Acute Epilepsy; Biochemical Parameters; Brain Histology; levetiracetam; Mesenchymal Stem Cells.

Epilepsy is a long-standing neurologic ailment with a series of conditions involving several kinds of seizures, syndromes and comorbidities¹. Seizures in epilepsy are frequent paroxysmal phenomena, manifested by stereotyped behavioral modifications that reflect the behind neural mechanisms of the disease². Also, epileptic seizures may arise after an acute injury of the central nervous system (CNS). The acute symptomatic or prompted seizures are considered as acute manifestations of the insult³, but they may not reoccur when the implied reason has been uncovered or the acute phase has elapsed⁴. The epileptic seizures arise in specific areas of the brain and they may persist enclosed confined to their area of origin (“focal” or “partial” seizures), or diffused to the whole cerebral hemispheres (“generalized” seizures)⁵.

Epilepsy is considered as one of the most prevalent critical brain situation, impacting over 70 million individuals all over the world. Its incidence has a bimodal distribution with the highest risk in infants and children⁶. More and more recent studies have referred to epilepsy as one of the most prevalent neurological disease, affecting 0.5-1% of the population globally of all ages, races, social classes, and geographical locations^{2,7}. The integrated incidence rate of epilepsy is 61.4 per 100.000 persons /year and this incidence is higher in lower middle – income nations than in higher income countries⁸. Commonly, the cause of epilepsy is unknown, but sometimes the origin of epilepsy may be associated with stroke, brain injury, brain tumors, or infections (such as meningitis, brain abscess, AIDS). Exposure to some particular drugs, atypical sodium/glucose levels in blood, or hyperpyrexia can be sometimes linked to impermanent cause of seizures⁹.

Currently, the antiepileptic drugs (AEDs) are the main treatment method for epileptic patients, and it was reported that, approximately two third of the epileptic seizures are controlled by the AEDs but about 30% of epilepsy cases are resistant to that treatment¹⁰. One of the most common second-generation anti-epileptic drugs is called levetiracetam (LEV) which was approved by FDA in 1999 as a drug for focal epilepsy in adults¹¹. Levetiracetam acts *via* binding to synaptic vesicle protein 2A, which minimizes the rate of calcium-dependent vesicular neurotransmitter liberation¹². Levetiracetam is characterized by rapid absorption and high bioavailability. Additionally, LEV is not metabolized by the cytochrome P450 system and it has a low protein binding rate, which indicates that there is no competition for protein binding sites with other drugs¹³. Kidneys excrete about two-third of the LEV dose in its native form owing to its efficiency and safety profile. However, the long use of the AEDs can lead to undesirable side effects¹⁰. The other available treatments for epilepsy like epilepsy surgery, and vagal nerve excitation are either not convenient in all patients or only slightly efficient and they may cause adverse events such as brain damage¹⁴. So, the current treatment options for epilepsy are not satisfactory, therefore there is an urgent need for replacement therapy having a therapeutic potential and minimal side effects.

Stem cell therapy has been proposed as a possible alternative and a potential breakthrough in the treatment of epilepsy^{15,16} with minimum adverse effects. Stem cells can treat both the biologic and psychiatric outcomes of epilepsy¹⁵. Mesenchymal stem cells (MSCs) have become the most employed adult stem cells in the regenerative medicine^{17,18} as they are able for self-renewal

and multilineage differentiation¹⁹. MSCs can be derived from adipose tissue (AD), bone marrow (BM), the umbilical cord and several other adult tissues²⁰. However, MSCs derived from adipose tissue (ADMSCs) and MSCs derived from bone marrow (BMMSCs) are the commonly MSCs used in the regenerative medicine owing to their ease of harvesting and conceivable autologous application, relatively low immunogenicity, good availability, and lack of ethical concerns^{16, 21}. Numerous researches demonstrated that BMMSCs share with ADMSCs in the morphological characteristics and the expression of cell surface antigen, but considerable biological variations have been detected regarding their proliferation rates^{22,23}, cytokines and chemokines receptor expression and differentiation capacities²⁴. A growing body of evidence demonstrated the efficacy of MSCs in the management of diverse neurodegenerative diseases like Alzheimer's disease, multiple sclerosis, stroke, and brain injury in animal models²⁵. Also, many investigators have considered MSCs as a hopeful curative option for the treatment of epilepsy^{26,27}. However, little information is available about the therapeutic potential of rodent MSCs in the acute phase of experimental epilepsy. Also, the combination of anti-epileptic drug and rodent ADMSCs or BMMSCs is not clearly described and entails to be clarified. Therefore, this approach was constructed to appraise the therapeutic outcome of a combined treatment of LEV and ADMSCs or BMMSCs against pilocarpine-induced acute phase of epilepsy in rats and to explore the possible mechanisms underlying this action. Thus, a set of relevant brain biochemical indices was investigated and histomorphological features of different brain areas were examined to achieve these purposes.

MATERIAL AND METHODS

Isolation and propagation of bone marrow mesenchymal stem cells

Bone marrow mesenchymal stem cells were isolated from the bone marrow of the tibias and femurs of two-month-old (120 g) male albino rats of *Wistar* strain. Briefly, three rats were sacrificed by decapitation and swiped with 70% ethyl alcohol followed by sterilized dissection. The tibias and femurs were excised and cleaned from muscles. Bone marrow was flushed out of the epiphysis

and suspended in Dulbecco's modified Eagle's medium [DMEM; Biowest, USA] supplemented with heparin (5 U/ml) to prevent clotting. Marrow in DMEM was centrifuged at 1800xg for 20 min to harvest the mononuclear cells. The cell pellet was resuspended with complete DMEM culture medium supplemented with 10% fetal bovine serum [FBS; Biowest, USA] and 1% Pencillin-Streptomycin [Biowest, USA]²⁸. Cell counting was done after addition of trypan blue dye in an automatic cell counter (Life Technologies, USA) and was then seeded in 25 cm² flask with a cell concentration of 1×10⁶ cells/ml. The culture flasks were maintained in a humid condition at 37°C with 5% CO₂. The culture medium was replaced every 3 days for removing of the non-adherent cells. Culture flasks were examined continuously using inverted microscope (Olympus, Japan).

When cultures approximately reached the confluence of 70-80% under the inverted optical microscope, the adherent cells were passaged using trypsin/ethylene diamine tetra acetic acid (EDTA) solution. Firstly, cultured cells were cleaned two times with phosphate buffer saline [PBS, Biowest, USA]. Then, trypsinization of the cells was done using 0.25% trypsin in 1 ml EDTA [Biowest, USA] for 5 min at 37°C. After centrifugation at 1800xg for 30 min, these cells were subcultured into 25 cm² flasks with complete culture medium. This continued culturing step led to a removal of non-adherent cells (non-BMMSCs) and this allows for the progressive purification of the BMMSCs. These cells were nominated passage 1 which were harvested using trypsin-EDTA solution and passaged to subculture again. When the adhesive cells in passage 2 reached the confluence of 70-80%, passage 3 BMMSCs was taken on.

Isolation and propagation of adipose mesenchymal stem cells

Adult male albino *Wistar* rats aging three months old and weighing between 250 and 300 g were sacrificed for fat harvesting. Adipose tissue was collected surgically from rat omentum and incised with scissors and cut into small fragments and carefully washed 3-4 times with equal volume of phosphate buffer saline PBS. Then, they were immersed in a solution of 0.2% collagenase type 1 (Sigma-Aldrich, USA) in PBS (200 mg/100 ml) then incubated for 60 min. at 37°C in shaker incubator and centrifuged at 1000xg for 10 min.

10% FBS DMEM medium was used for inactivation of collagenase, then the mixture was transferred into 50 ml falcon tube and centrifuged at 1000xg for 10 min at room temperature to isolate the oil and the residual fat lobules from the stromal vascular fraction (SVF). Floating adipocytes, lipids and liquids were aspirated leaving SVF pellet which was processed with 160 mM NH₄CL at room temperature for 10 min to lyse RBCs. Then, centrifugation of this mixture was done at 400xg for 10 min and employed for a Ficoll gradient (Sigma-Aldrich, USA) to refine the mononucleated cells. Centrifugation at 1000xg for 30 min was taken place and gradient separated cells were cleaned two times with PBS and centrifuged at 400xg for 10 min between each wash. Cells pellet was resuspended in PBS, filtered and centrifuged at 400xg for 10 min. The isolated stromal vascular fraction was filtered, centrifuged and then the pellet was resuspended in DMEM containing 10% FBS and 1% pencillin-streptomycin solution²⁹. Cell counting was done after addition of trypan blue dye in an automatic cell counter and was then seeded in 25 cm² flask with a cell concentration of 1×10⁶ cells/ml.

After 24 hours of culturing, the non-adherent cells in the flask were discarded by PBS washing. The cultures were preserved at sub-confluent levels at 37 °C incubator with 5% CO₂ and passaged with trypsin /EDTA, when demanded as mentioned previously.

Identification of MSCs

Cell morphology

The resulting cultured cells were investigated morphologically under the inverted optical microscope (Olympus, Japan) to ensure the identity of the MSCs.

Surface markers profile

Flow cytometry analysis was used for detection of the cell surface markers of MSCs (CD90, CD14 and CD45) to assure whether the derived AD-MSCs and BM-MSCs preserve their phenotype after culture expansion³⁰. The FITC conjugated-CD90 antibody was purchased from Immunotech SAS, (France) whereas, the PE-conjugated CD14 and PE-conjugated CD45 antibodies were obtained from R & D systems, (UK and Germany respectively). Briefly, the incubation of the cells with the antibody against each of the surface markers was done for 30 min at 4 °C in case of CD90 and 10 min at 4 °C for CD14 and CD45

followed by flow cytometry analysis (Beckman Coulter Elite XL, USA).

Staining of MSCs with PKH26 dye for Verification of Accommodation

The third passage of MSCs was collected and marked with PKH26 dye by using the PKH26 Fluorescent Cell Linker kits (Sigma-Aldrich, USA) following to the manufacturer's manual to clarify their homing in acute epileptic rat brain.

Animal's handling

Fifty-six male albino *Wistar* rats weighing 220-240 g were procured from the Unit of the Animal Care of the National Research Centre, Giza, Egypt and acclimated in a definite space when the temperature (25 ± 1) and humidity (55%). Rats were monitored continuously with a 12 h light/dark cycle at the National Research Centre and they had free access to water and food. Rats were cared for in accordance with the guidelines for the animal experiments which were approved by the Ethical Committee of Medical Research at the National Research Centre, Giza, Egypt (Approval No 14/018).

Experimental procedures

Animal grouping

In this study, the rats were classified into 7 equal groups (8 rats/group) as follows:

Group 1: Control group, healthy normal rats.

Group 2: Epileptic group; the rats were injected intraperitoneally with methylscopolamine (1mg/kg b.wt)³¹ 30 min prior the intraperitoneal pilocarpine injection (380 mg/kg b.wt)³². After that, all rats received two doses of diazepam separated by one hour (each 4 mg/kg b.wt orally)³³ 30 min after seizure onset. These rats were sacrificed after 24 hours from final administration³⁴.

Group (3): Epileptic group treated with levetiracetam (Epileptic + LEV); the rats were administered orally with levetiracetam 300 mg / kg b.wt., after induction of acute phase of epilepsy, (24 hours)³⁵.

Group (4): Epileptic group treated with ADMSCs (Epileptic + ADMSCs); the rats were intravenously infused with a single dose of ADMSCs (3 × 10⁶ cells/rat)³⁶, after induction of acute phase of epilepsy.

Group (5): Epileptic group treated with BMMSCs (Epileptic + BMMSCs), the rats were intravenously infused with a single dose of BMMSCs (3 × 10⁶ cells/rat)³⁷, after induction of acute phase epilepsy.

Group (6): Epileptic group treated with ADMSCs and LEV (Epileptic + ADMSCs + LEV), the rats were intravenously infused with a single dose of ADMSCs (3×10^6 cells/rat) then they were orally administered with LEV 300 mg/kg b.wt./day for 12 weeks³⁵ after induction of acute phase of epilepsy.

Epileptic rats

Healthy rats

Group (7): Epileptic group treated with BMMSCs and LEV (Epileptic + BMMSCs + LEV), the rats were intravenously infused with a single dose of BMMSCs (3×10^6 cells/rat), then they were orally administered with LEV 300 mg/kg b.wt./day for 12 weeks³⁵ after induction of acute phase of epilepsy.

For MSCs transplantation, the epileptic rats were deeply anesthetized *via* isoflurane (1–3%) inhalation and MSCs either isolated from adipose tissue or bone marrow were suspended in 100 μ l PBS before infusion and then slowly infused into the tail vein in 5 min with a 27G needle. The needle was maintained in tail vein for other 5 min to prevent regurgitation and then withdrawn.

Mean Electric Shock (MES) Test

This test was principally realized according to Kitano *et al.*³⁸ and Krall *et al.*³⁹. All rats in the different studied groups were tested by the mean electric shock test to prove the presence of strong seizures in the epileptic group and the modulation of these seizures after treatments. An increased electric current was applied via an ear electrode, initial current of 1 mA, increment of 0.1 mA/0.2 s and at 50 Hz (Ugo Basil, ECT Unit, 57800, Italy). Tonic extension of the hind limbs is taken as the end point. The mean convulsion threshold was considered as the mean of the maximal electric current which could be tolerated by the animal at the end point⁴⁰. The obtained values represent a mean of that of eight rats (\pm standard error). The rats in the control group were also subjected to this test for comparison.

Brain tissue sampling

After the period of 12 weeks, the end of the experimental process, the rats were fasted for about 10 hours and then, they were submitted to anesthesia to be sacrificed by decapitation and the whole brain of each rat was quickly and carefully dissected, cleaned gently with isotonic saline and blotted dry. Then, each brain was partitioned into two longitudinally equal halves,

the right half was immediately stored at -80°C pending the biochemical determinations while, the left half was fixed in formalin buffer (10%) for histomorphological investigation.

Preparation of brain homogenate

The first half of each brain was weighed and homogenized to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose, pH: 7.4⁴¹. The homogenates were centrifuged at 1800xg for 10 min in cooling centrifuge at 4 $^\circ\text{C}$. The supernatants (10%) were stored in aliquots in -20°C and used for the biochemical determinations [gamma aminobutyric acid (GABA), glutamate, dopamine, basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α)].

Biochemical determinations

Brain gamma aminobutyric acid (GABA), glutamate, dopamine, basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) levels were determined in brain homogenate by ELISA using kits obtained from Glory Science Co., USA, according to the recommendation of the manufacturer provided with the kits.

Histomorphological procedure

Twenty-four hours after fixation of brain tissue in 10% formalin buffer, washing was done in tap water, then, serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in molten paraffin wax at 56 $^\circ\text{C}$ in hot air oven for twenty-four hours. Paraffin wax tissue blocks were prepared for sectioning at 4 microns thick slices by rotary microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for routine examination through the optical microscope (Leica DM2500 & DM2500 LED, Germany)⁴².

Statistics

In the current investigation, all results were expressed as Mean \pm standard error (SE) of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program version 20, followed by least significant difference (LSD) to compare the significance between groups. The

difference was considered significant when P value was <0.05.

RESULTS

Microscopic photographs of MSCs

The photomicrographs presented in Fig. (1) showed the morphology of cells derived from rat adipose tissue (A) and bone marrow (B) at the third passage. Images indicate that MSCs isolated from rat adipose tissue and bone marrow have obvious characteristic of spindle-shaped as fibroblast-like morphology cells.

Surface marker profile of MSCs

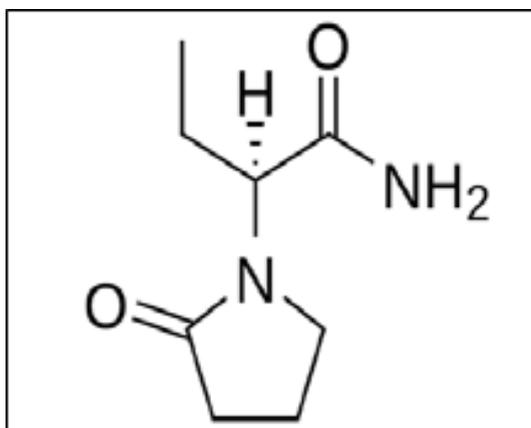
Flow cytometry analysis showed that the isolated ADMSCs are positive for CD90 (88.1%), negative for CD14 (4.20 %) and CD45 (6.70%) (Fig. 2A, B, C). Also, the results of flow cytometry analysis revealed that the isolated BMMSCs are positive for CD90 (86.3%), negative for CD14 (6.59%) and CD45 (7.50%) (Fig. 2D, E, F).

Homing manifestation using PKH26 Dye

Fluorescence microscope investigation of brain tissue sections of rats treated with ADMSCs (Fig. 3 (A)), BMMSCs (Fig. 3 (B)), ADMSCs + LEV (Fig. 3 (C)) and BMMSCs + LEV (Fig. 3 (D)) displayed that the MSCs are stained with PKH26 dye. They appeared in the different brain areas of ADMSCs, BMMSCs, ADMSCs + LEV and BMMSCs + LEV treated rats making sure of MSCs homing in the damaged areas of the epileptic rat brain.

Behavioral assessment

After pilocarpine injection, the rats were closely observed and the behavioral changes were



Scheme 1. Chemical structure of levetiracetam (LEV)

recorded. During the first 5 minutes after pilocarpine administration, the rats evolved diarrhea and other manifestations of cholinergic excitation. After 15-20 minutes following pilocarpine administration, the rats displayed head bobbing, scratching, chewing, and exploratory behavior. Repeated seizures started around 40-50 minutes after

Table 1. Influence of treatment with rat BMMSc or rat ADMSc with or without LEV on the MES values of acute epileptic rat model

Group Number	Groups	(MES) values
Group 1	Control	11.0±0.087
Group 2	Epileptic	4.3±0.61* (-60.9%) ^a
Group 3	Epileptic + LEV	10.8±0.40** (-1.8) ^a (151%) ^b
Group 4	Epileptic + ADMSc	10.0±0.71** (132.5%) ^b (20%) ^f (42.8%) ^g
Group 5	Epileptic + BMMSc	7.0±0.26** (62.9%) ^b (-2.7%) ^c
Group 6	Epileptic + ADMSc + LEV	8.3±0.33** (93%) ^b (-23%) ^c
Group 7	Epileptic + BMMSc + LEV	7.2±0.37** (67.44%) ^b (-33%) ^c (-13%) ^d

MES: Mean electric shock.

LEV: Levetiracetam

ADMSCs: Adipose tissue- derived mesenchymal stem cells

BMMSCs: Bone marrow-derived mesenchymal stem cells

Data were represented as Mean ± SE

Data were analyzed using One-way ANOVA followed by Tukey's multiple comparisons test

Significant change at p<0.05 between epileptic group and the control group*

Significant change at p<0.05 between epileptic group and all the treated groups**

a Percent of change between epileptic group and the control group

b Percent of change between epileptic group and all the treated groups

c Percent of change between Levetiracetam (LEV) and ADMSCs +LEV as well as BMMSCs + LEV

d Percent of change between ADMSCs +LEV and BMMSCs + LEV

e Percent of change between BMMSCs + LEV and BMMSCs

f Percent of change between ADMSCs +LEV and ADMSCs

g Percent of change between BMMSCs and ADMSCs

pilocarpine administration, which lasted for 1-2 days, this period was described as the acute phase of epilepsy^{43,44}.

Mean Electric Shock (MES) recordings

The results of Table (1) visualized a significant ($p < 0.05$) decrease in the MES test of the epileptic rats in comparison with that of the control counterparts. Whereas, the MES test recorded significant ($p < 0.05$) enhancement, in the epileptic rats treated with LEV, or ADMSCs or BMMSCs, respectively, versus rats administered pilocarpine alone (epileptic rats). Also, the MES test registered significant ($p < 0.05$) elevation in the epileptic rats treated with either ADMSCs + LEV or BMMSCs + LEV, when compared to the results of the rats administered pilocarpine alone.

The data in Table (1) also revealed that there is a great difference in MES records

between the group of the epileptic rats treated with ADMSCs and those treated with BMMSCs; the leading effect has been observed in the group of epileptic rats treated with ADMSCs. However, the groups of epileptic rats treated with of ADMSCs + LEV or with BMMSCs + LEV showed an almost similar effect in MES recordings in (Table 1). The preferable outcomes have been demonstrated in the epileptic rats treated with ADMSCs + LEV (Table 1).

Biochemical outcomes

The data in Table (2) showed that during the acute phase of epilepsy, 24 hours after the administration of pilocarpine, the level of brain GABA of the epileptic rats decreased ($p < 0.05$) significantly, while the levels of brain glutamate and dopamine increased ($p < 0.05$) significantly when compared to the findings of the control

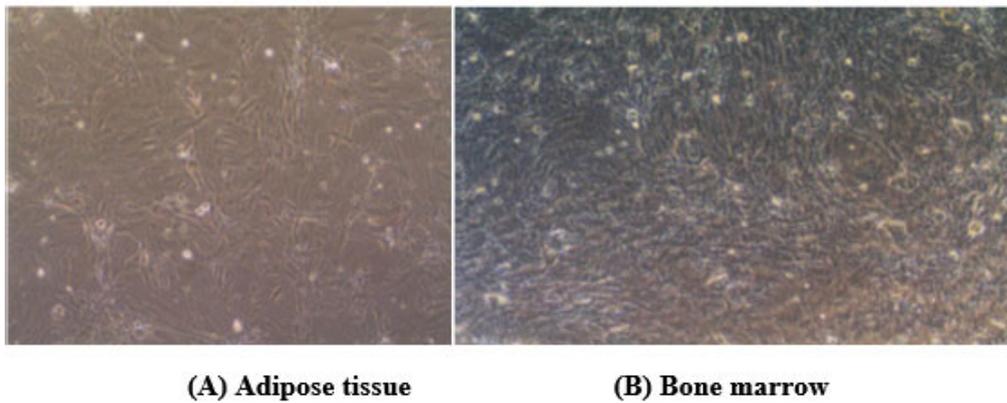
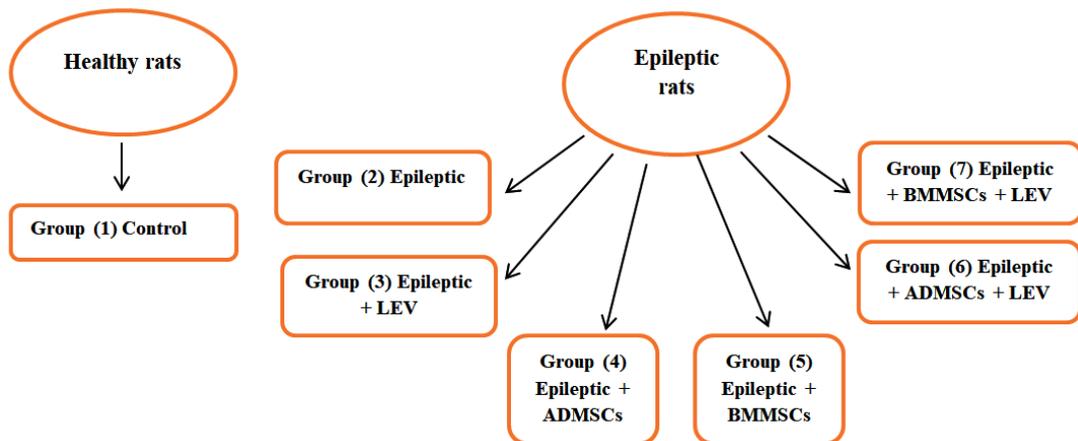


Fig. 1. Morphology of mesenchymal stem cells (MSCs), third passage, under the inverted optical Microscope (Olympus, Japan) showing spindle shape feature. (X 100)



Scheme 2. Graphical representation of the experimental groups

Table 2. Influence of treatment with rat BMMSc or rat ADMSc with or without LEV on brain GABA, glutamate and dopamine levels of acute epileptic rat model

Group Number	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Group / parameters	Control	Epileptic	Epileptic + LEV	Epileptic + ADMScs	Epileptic + BMMScs	Epileptic + ADMScs + LEV	Epileptic + BMMSc + LEV
GABA $\mu\text{mol/g}$	4.18 \pm 0.50	1.26 \pm 0.21* (-69.86%) ^a	3.46 \pm 0.41** (-17.22%) ^a (+174.60%) ^b	3.24 \pm 0.61** (-22.49%) ^a (+157.14%) ^b	2.43 \pm 0.32 (-41.87%) ^a (+92.87%) ^b (-25.0%) ^g	3.18 \pm 0.08** (-23.92%) ^a (+152.38%) ^b (-8.09%) ^c (-1.85%) ^f	2.59 \pm 0.26 (-38.04%) ^a (+105.55%) ^b (-25.14%) ^c (+6.58%) ^e (-18.59%) ^d
Glutamate ($\mu\text{mol/g}$)	5.30 \pm 0.81	11.24 \pm 1.48* (+112.07%) ^a	5.71 \pm 1.14** (+7.73%) ^a (-49.20%) ^b	5.97 \pm 0.48** (+12.64%) ^a (-46.89%) ^b	7.13 \pm 0.55** (+34.53%) ^a (-36.56%) ^b (+19.44%) ^g	6.14 \pm 0.64** (+15.85%) ^a (-45.37%) ^b (+7.53%) ^c (+2.45%) ^f	6.69 \pm 0.47** (+26.3%) ^a (-40.48%) ^b (+17.16%) ^c (+6.17%) ^e (+8.96%) ^d
Dopamine (ng/g)	984 \pm 108	1911 \pm 238* (+94.21%) ^a	1051 \pm 159** (+6.81%) ^a (-45.0%) ^b	1136 \pm 89.4** (+15.45%) ^a (-40.55%) ^b	1248 \pm 110** (+26.83%) ^a (-34.69%) ^b (+9.86%) ^g	1163 \pm 42.3** (+18.19%) ^a (-39.14%) ^b (+10.65%) ^c (+2.38%) ^f	1189 \pm 90.2** (+20.83%) ^a (-37.78%) ^b (+13.13%) ^c (-4.73%) ^e (-2.33%) ^d

GABA: Gamma aminobutyric acid

LEV: Levetiracetam

ADMScs: Adipose tissue-derived mesenchymal stem cells

BMMScs: Bone marrow-derived mesenchymal stem cells

Data were represented as Mean \pm SE

Data were analyzed using One-way ANOVA followed by Tukey's multiple comparisons test

Significant change at p<0.05 between epileptic group and the control group*

Significant change at p<0.05 between epileptic group and all the treated groups**

a Percent of change between epileptic group and the control group

b Percent of change between epileptic group and all the treated groups

c Percent of change between Levetiracetam (LEV) and ADMScs +LEV as well as BMMScs+ LEV

d Percent of change between BMMScs+LEV and BMMScs+ LEV

e Percent of change between ADMScs +LEV and BMMScs

f Percent of change between ADMScs +LEV and ADMScs

g Percent of change between BMMScs and ADMScs

rats. Upon treatment of epileptic rats with LEV or ADMSCs or BMMSCs, the level of brain GABA increased ($p < 0.05$) significantly, with respect to the findings of the epileptic rats. Significant decrease in the level of brain glutamate, as well as in the level of brain dopamine were recorded in the same treated groups, in comparison with the data of the epileptic group. It could be observed that ADMSCs showed an almost similar effect on the brain neurotransmitters like that of the antiepileptic drug Levetiracetam, and the effect of BMMSCs was lower than that of the ADMSCs.

Table (2) showed also that treatment of the epileptic rats with ADMSCs + LEV or BMMSCs + LEV yields significant elevation ($p < 0.05$) in the level of brain GABA and, and significant decrease in the level of brain glutamate respectively), as well as, in the level of brain dopamine, relative to the results of the epileptic rats. It could be observed that the effect the combined treatment

of ADMSCs + LEV or BMMSCs + LEV was close to the effect of either ADMSCs or BMMSCs alone regarding brain neurotransmitters levels, since insignificant changes were recorded in the levels of brain GABA, glutamate and dopamine after administration of either ADMSCs + LEV or BMMSCs + LEV, in comparison with the findings of either ADMSCs or BMMSCs alone. The effect of ADMSCs + LEV on brain GABA, glutamate and dopamine levels was higher than the effect of BMMSCs + LEV.

Table (3) showed that in the acute phase of epilepsy, 24 hours after pilocarpine administration (epileptic group), the level of brain bFGF and brain BDNF increased ($p < 0.05$) significantly, versus the result of the control group. When the three groups of epileptic rats were treated with LEV or ADMSCs or BMMSCs, a significant reduction was recorded in the levels of brain bFGF as well as in the level of brain BDNF, when compared with the results

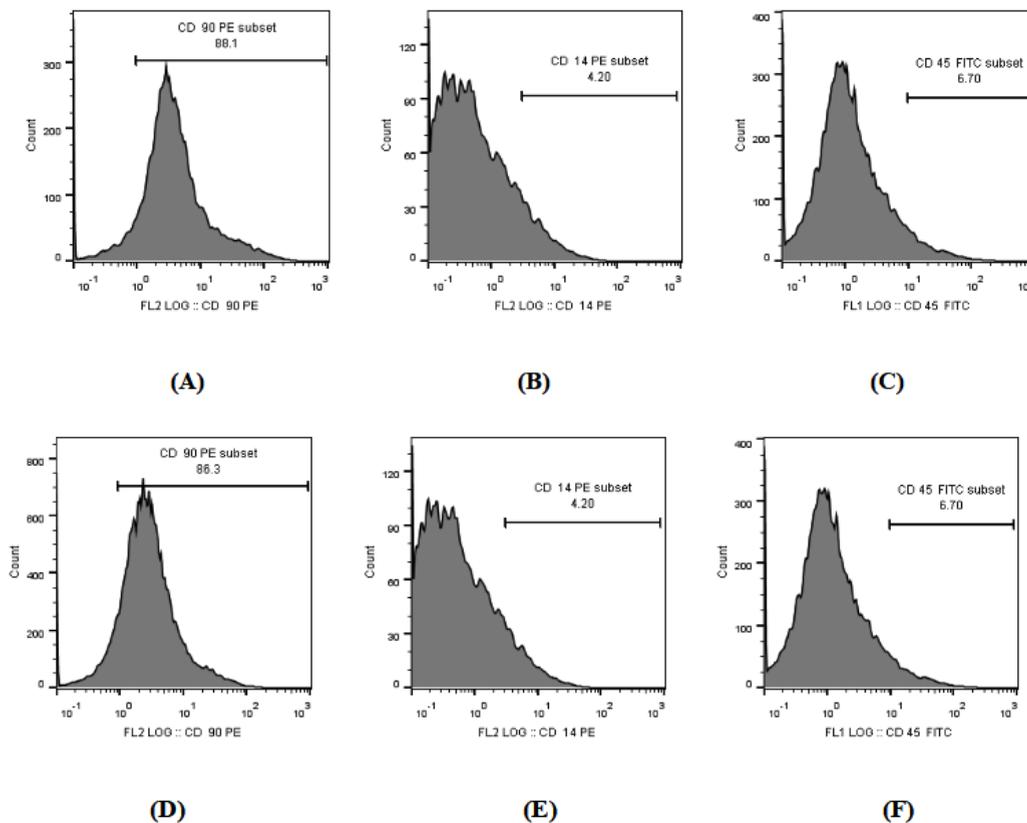


Fig. 2. Flow cytometry analysis of CD surface marker of ADMSCs (CD90 (A), CD14 (B) and CD45 (C)) and BMMSCs (CD90 (D), CD14 (E) and CD45 (F))

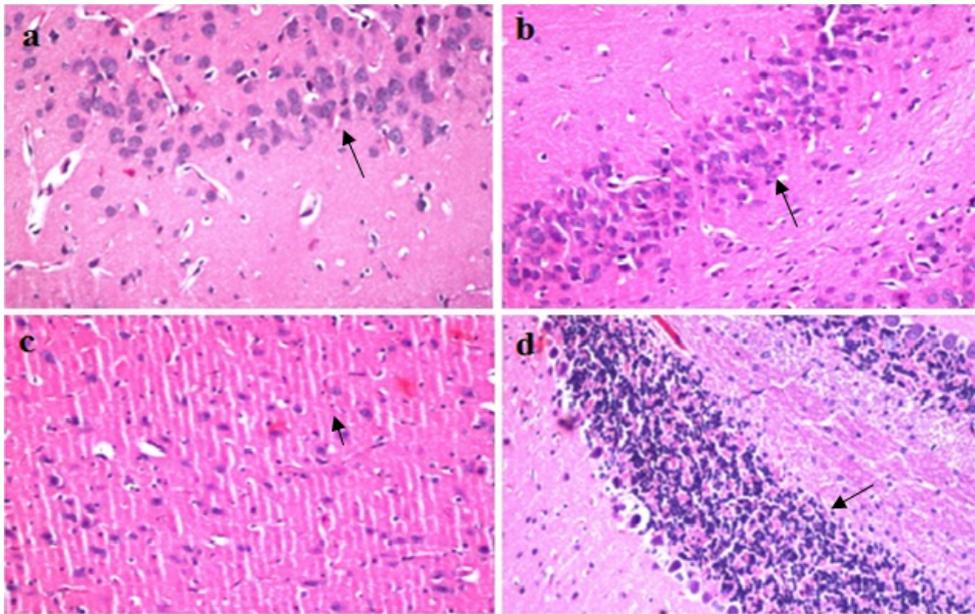


Fig. 4. Optical photomicrograph of brain tissue section of rat in the control group showing: (a) normal histoarchitecture of the cerebral cortex and covering meninges (arrow), (b) normal histoarchitecture of subiculum neuronal cells (arrow) in the hippocampus, (c) normal histoarchitecture of striatum (arrow) and (d) normal histoarchitecture in neurons (arrow) of cerebellum (Optical microscope (Leica DM2500 & DM2500 LED, Germany)). (H&EX40)

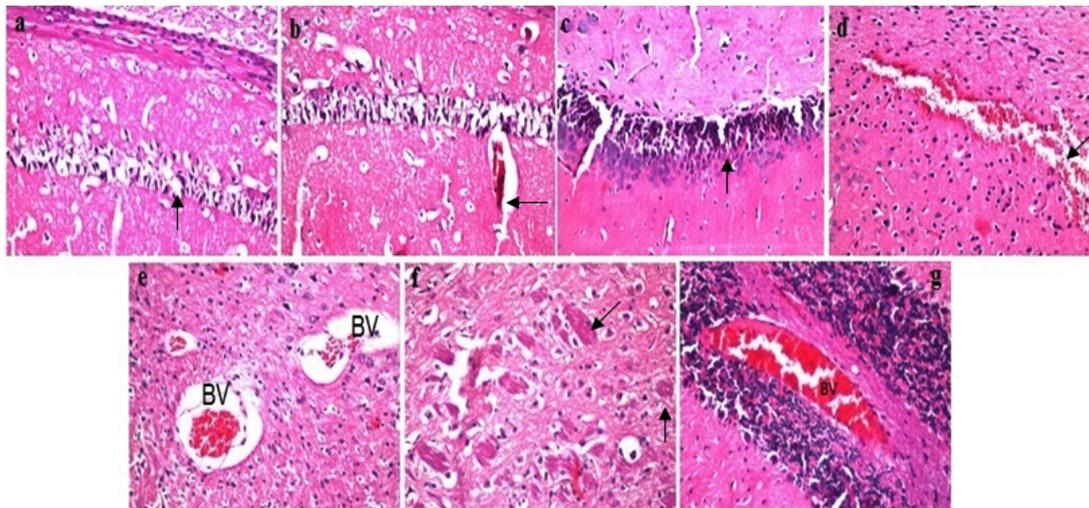


Fig. 5. Optical photomicrograph of brain tissue sections of rat in the acute phase of epileptic group showing: (a) degeneration and atrophy in the neuronal cells (arrow) of subiculum in hippocampus, (b) degeneration and congestion blood vessels (arrow) in the neuronal cells of subiculum of hippocampus, (c) degeneration and atrophy in the neuronal cells (arrow) of fascia dentata in hippocampus, (d) focal haemorrhage (arrow) in the striatum of the cerebrum, (e) congestion in blood vessels (BV) in the striatum of the cerebrum, (f) focal eosinophilic plaques formation (arrow) in the striatum of the cerebrum and (g) congestion in blood vessels (BV) of the cerebellum (Optical microscope (Leica DM2500 & DM2500 LED, Germany)). (H&EX40)

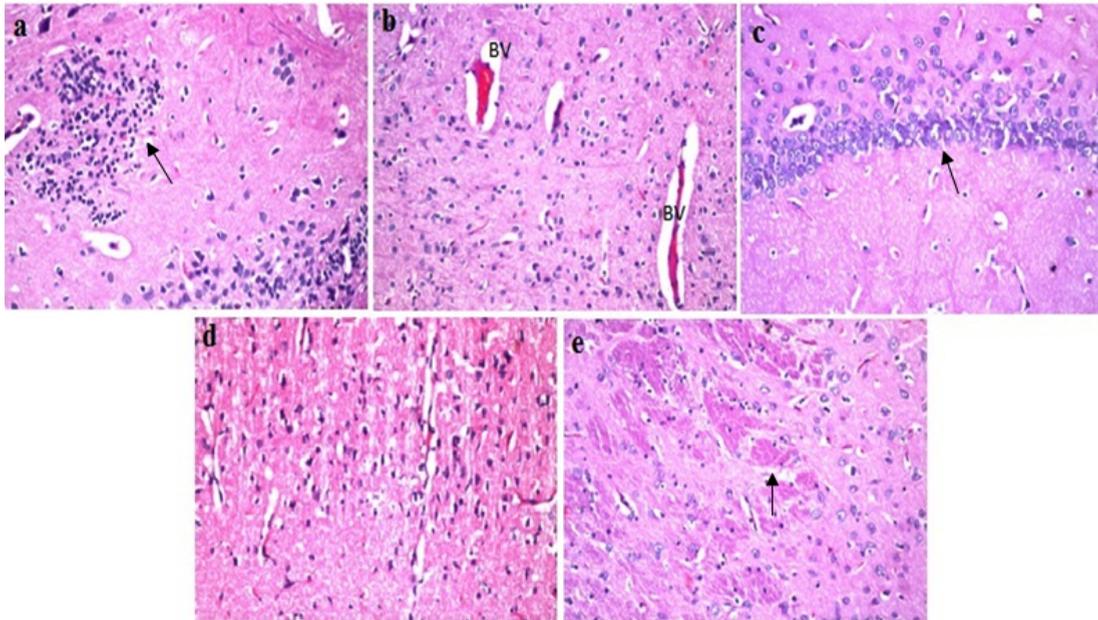


Fig. 6. Optical photomicrograph of brain tissue sections of rat in the group treated with Levetiracetam showing: (a) focal gliosis (arrow) of the cerebral cortex, (b) congestion in blood vessels (BV) of cerebral cortex, (c) normal histoarchitecture of subiculum neurons (arrow) in hippocampus, (d) neuronal degeneration in the striatum and (e) focal eosinophilic plaques formation (arrow) in the striatum (Optical microscope (Leica DM2500 & DM2500 LED, Germany)). (H&EX40)

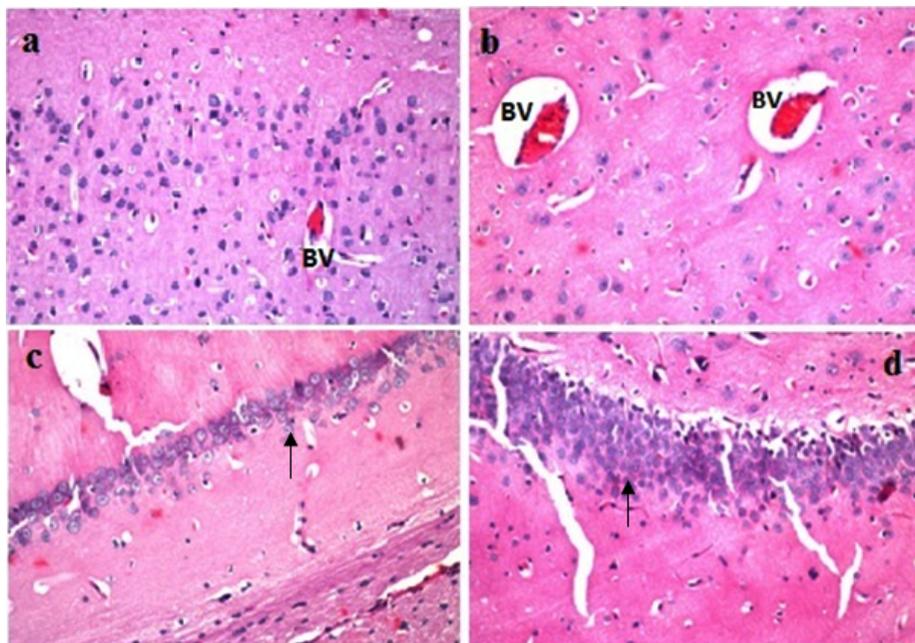


Fig. 7. Optical photomicrograph of brain tissue sections of rat in the group treated with ADMSCs showing: (a) congestion in the blood vessels (BV) of cerebral cortex, (b) congestion in the blood vessels (BV) of cerebral cortex, (c) normal histoarchitecture of subiculum neurons (arrow) of hippocampus and (d) normal histoarchitecture of fascia dentata (arrow) in hippocampus (Optical microscope (Leica DM2500 & DM2500 LED, Germany)). (H&EX40)

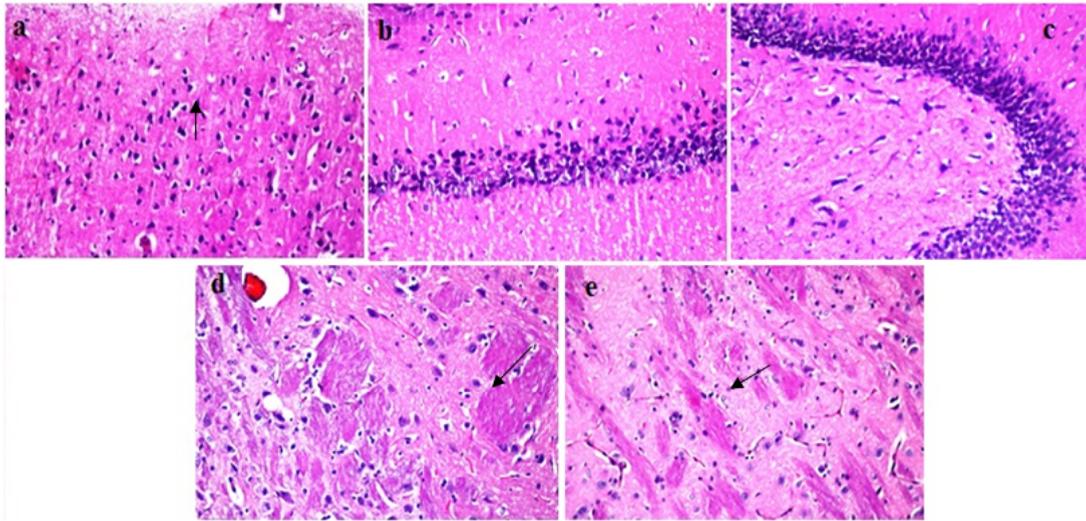


Fig. 8. Optical photomicrograph of brain tissue sections of rat in the group treated with BMMSCs showing: (a) nuclear pyknosis in neuronal cells (arrow) of cerebral cortex, (b) nuclear pyknosis in neuronal cells of subiculum of hippocampus, (c) nuclear pyknosis in neuronal cells of fascia dentata of hippocampus, (d) focal eosinophilic plaques formation (arrow) in the striatum and (e) focal eosinophilic plaques formation (arrow) in the striatum (Optical microscope (Leica DM2500 & DM2500 LED, Germany)). (H&EX40)

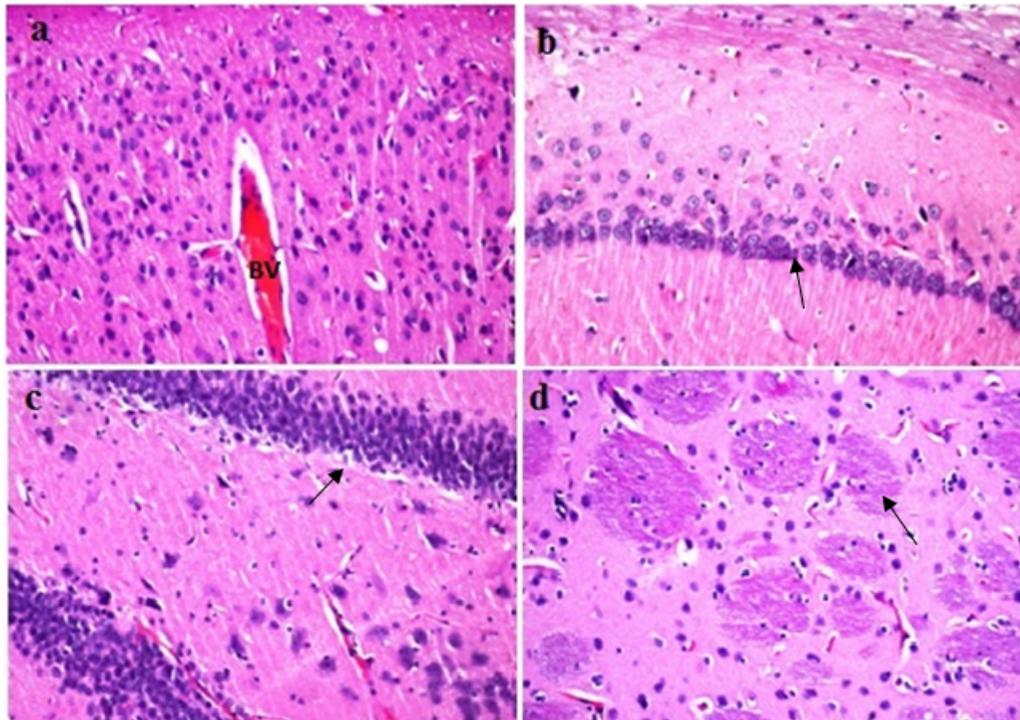


Fig. 9. Optical photomicrograph of brain tissue sections of rat in the group treated with ADMSCs +LEV showing: (a) nuclear pyknosis in neuronal cells of cerebral cortex, (b) normal histoarchitecture of subiculum neurons (arrow) in hippocampus, (c) normal histoarchitecture of fascia dentata and hilus neurons (arrow) in hippocampus and (d) focal eosinophilic plaques formation (arrow) in the striatum (Optical microscope (Leica DM2500 & DM2500 LED, Germany)). (H&EX40)

increased ($p < 0.05$) significantly, when compared to the results of the control group. When the epileptic groups were treated with either LEV, ADMSCs or BMMSCs, significant decreases ($p < 0.05$) were recorded in the level of brain IL-6, and also in the level of brain TNF α versus the results of the epileptic group. It could be detected that the two types of MSCs showed roughly similar effect on the level of brain IL-6 and brain TNF- α like that of antiepileptic drug Levetiracetam, and the effect of ADMSCs on brain IL-6 and brain TNF- α was higher than that of BMMSCs.

After 24 hours of administration of pilocarpine (acute phase of epilepsy), the treatment with either ADMSCs + LEV or BMMSCs + LEV produced significant decrease ($P < 0.05$) in the level of brain IL-6, respectively, and also in the level of brain TNF- α , in respect with the findings of the epileptic group (Table 4). Thus, there is an insignificant difference ($P < 0.05$) between the effect of ADMSCs + LEV and BMMSCs + LEV on brain IL-6 and brain TNF- α levels.

Histomorphological observations

The histomorphological description of brain tissue section of rat in the control group revealed normal histological structure of the cerebral cortex and covering meninges, subiculum neuronal cells in the hippocampus as well as striatum and neurons of cerebellum (Fig. 4). While, histomorphological examination of brain tissue section of rat in the epileptic group showed degeneration and atrophy in the neuronal cells of subiculum in hippocampus, degeneration and congestion blood vessels in the neuronal cells of subiculum of hippocampus, degeneration and atrophy in the neuronal cells of fascia dentata in hippocampus, focal haemorrhage in the striatum of the cerebrum, congestion in blood vessels in the striatum of the cerebrum, focal eosinophilic plaques formation in the striatum of the cerebrum and congestion in blood vessels of the cerebellum (Fig. 5).

Histomorphological examination of brain tissue section of rat in the group treated

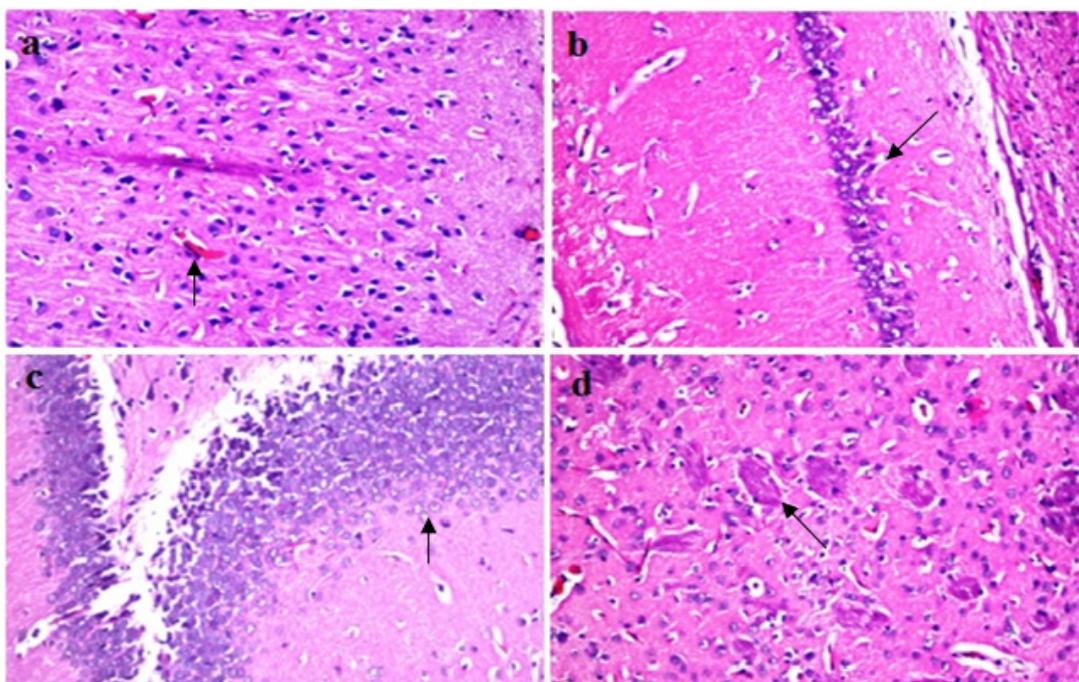


Fig. 10. Optical photomicrograph of brain tissue sections of rat in the group treated with BMMSCs + LEV showing: (a) congestion in cerebral cortical blood vessels (arrow), (b) normal histoarchitecture of neurons (arrow) of subiculum in hippocampus, (c) normal histoarchitecture of fascia dentata and hilus neurons (arrow) in hippocampus and (d) focal eosinophilic plaques formation (arrow) in the striatum (Optical microscope (Leica DM2500 & DM2500 LED, Germany)). (H&EX40)

Table 3. Influence of treatment with rat BMMSc or rat ADMSc with or without LEV on brain bFGF and BDNF levels of acute epileptic rat model

Group Number	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Group /parameters	Control	Epileptic	Epileptic + LEV	Epileptic + ADMScs	Epileptic + BMMScs	Epileptic + ADMScs + LEV	Epileptic + BMMSc + LEV
bFGF (ng/g)	198±17	462±18* (+133.33%) ^a	188±4.18** (-5.05%) ^a (-59.31%) ^b	177±3.26** (-10.61%) ^a (-61.69%) ^b	131 ±2.03 (-33.84%) ^a (-71.64%) ^b (-25.10%) ^g	161±16** (-18.69%) ^a (-65.15%) ^b (-14.36%) ^c (-9.04%) ^f	140 ±9.48** (-29.29%) ^a (-69.70%) ^b (-25.53%) ^c (+6%.87%) ^e (-13.04%) ^d
BDNF(pg/g)	646±28	913±64* (+41.33%) ^a	539±41** (-16.65%) ^a (-40.96%) ^b	537±10** (-16.87%) ^a (-41.18%) ^b	402±23** (-37.77%) ^a (-55.97%) ^b (-43.76%) ^g	531±55** (-17.80%) ^a (-41.84%) ^b (-1.48%) ^c (-1.12%) ^f	419±12** (-35.14%) ^a (-54.11%) ^b (-22.26%) ^c (+4.23%) ^e (-21.09%) ^d

bFGF : basic fibroblast growth factor

BDNF: Brian derived neurotrophic factor

LEV: Levetiracetam

ADMScs: Adipose tissue- derived mesenchymal stem cells

BMMScs: Bone marrow-derived mesenchymal stem cells

Data were represented as Mean±SE

Data were analyzed using One-way ANOVA followed by Tukey's multiple comparisons test

Significant change at p< 0.05 between epileptic group and the control group*

Significant change at p< 0.05 between epileptic group and all the treated groups**

a Percent of change between epileptic group and the control group

b Percent of change between epileptic group and all the treated groups

c Percent of change between Levetiracetam (LEV) and ADMScs +LEV as well as BMMScs+ LEV

d Percent of change between ADMScs +LEV and BMMScs+ LEV

e Percent of change between BMMScs+ LEV and BMMScs

f Percent of change between ADMScs +LEV and ADMScs

g Percent of change between BMMScs and ADMScs

Table 4. Influence of treatment with rat BMMSc or rat ADMSc with or without LEV on brain IL-6 and TNF- α levels of acute epileptic rat model

Group Number Group /parameters	Group 1 Control	Group 2 Epileptic	Group 3 Epileptic + LEV	Group 4 Epileptic + ADMScs	Group 5 Epileptic + BMMScs	Group 6 Epileptic + ADMScs + LEV	Group 7 Epileptic + BMMSc + LEV
IL-6 (ng/g)	0.087±0.021	0.232±0.029* (+166.67%) ^a	0.105±0.023** (+20.69%) ^a (-54.74%) ^b	0.124±0.026** (+42.53%) ^a (-46.55%) ^b	0.142±0.016* (+63.22%) ^a (-38.79%) ^b (+14.52%) ^g	0.130±0.012** (+49.42%) ^a (-43%) ^b (23.80%) ^c (+4.84%) ^f	0.133±0.018** (+52.87%) ^a (-42.69%) ^b (26.66%) ^c (+2.31%) ^e (+2.31%) ^d
TNF- α (ng/g)	0.248±0.029	0.514±0.066* (+107.6%) ^a	0.265±0.073** (+6.85%) ^a (-48%.44) ^b	0.347±0.025** (+39.92%) ^a (-32.50%) ^b	0.403±0.017 (+62..5%) ^a (-21.59%) ^b (+16.14%) ^g	0.375±0.004 (+51.21%) ^a (-27.04%) ^b (41.50%) ^c (+8.07%) ^f	0.385±0.072 (+55.24%) ^a (-25.4%) ^b (45.28%) ^c (-4.23%) ^e (+2.67%) ^d

IL-6: Interleukin -6

TNF- α : Tumor necrosis factor- α

LEV: Levetiracetam

ADMScs: Adipose tissue- derived mesenchymal stem cells

BMMScs: Bone marrow-derived mesenchymal stem cells

Data were represented as Mean±SE

Data were analyzed using One-way ANOVA followed by Tukey's multiple comparisons test

*Significant change at $p < 0.05$ between epileptic group and the control group** Significant change at $p < 0.05$ between epileptic group and all the treated groups

a Percent of change between epileptic group and the control group

b Percent of change between epileptic group and all the treated groups

c Percent of change between Levetiracetam (LEV) and ADMScs +LEV as well as BMMScs+ LEV

d Percent of change between ADMScs +LEV and BMMScs+ LEV

e Percent of change between BMMScs+ LEV and BMMScs

f Percent of change between ADMScs +LEV and ADMScs

with LEV showed focal gliosis of the cerebral cortex, congestion in blood vessels of cerebral cortex, normal histological structure of subiculum neurons in hippocampus, neuronal degeneration in the striatum and focal eosinophilic plaques formation in the striatum (Fig. 6). Furthermore, histomorphological examination of brain tissue sections of rat in the group treated with ADMSCs showed congestion in the blood vessels of cerebral cortex, congestion in the blood vessels of cerebral cortex, normal histological structure of subiculum neurons of hippocampus and normal histological structure of fascia dentata in hippocampus (Fig. 7). Histomorphological examination of brain tissue section of rat in the group treated with BMMSCs showed nuclear pyknosis in neuronal cells of cerebral cortex and subiculum of hippocampus as well as fascia dentata of hippocampus. In addition to, focal eosinophilic plaques formation in the striatum (Fig. 8). Moreover, histomorphological examination of brain tissue section of rat in the group treated with ADMSCs + LEV showed nuclear pyknosis in neuronal cells of cerebral cortex, normal histological structure of subiculum neurons in hippocampus, normal histological structure of fascia dentata and hilus neurons in hippocampus and focal eosinophilic plaques formation in the striatum (Fig. 9). Finally, histomorphological examination of brain tissue section of rat in the group treated with BMMSCs + LEV showed congestion in cerebral cortical blood vessels, normal histological structure of neurons of subiculum, fascia dentata and hilus neurons in hippocampus and focal eosinophilic plaques formation in the striatum (Fig. 10).

DISCUSSION

The current approach was undertaken to investigate the potent therapeutic effect of rat ADMSCs and BMMSCs on a pilocarpine rat model of acute epilepsy. A comparison among the effect of the antiepileptic drug, LEV, ADMSCs and BMMSCs on the relevant brain biochemical markers in the acute phase of epilepsy was investigated. Also, the effect of co-administration of the two types of MSCs with LEV was evaluated.

Indeed, the antiepileptic drugs fail to well-control seizures in approximately 30% of epilepsy patients and stem cell grafting has been

shown to be a promising alternative approach for a better treatment of epilepsy⁴⁵. There are two proposed mechanisms of stem cell transplantation in the treatment of epilepsy. In one mechanism, the transplanted stem cells may replace the cells lost by trans-differentiation. Stem cells possess self-renewal properties, and the ability to differentiate into different cell types, which may replace lost cells or specific neurons during epilepsy, such as inhibitory interneurons⁴⁶. The other mechanism relies on the neuroprotective or anti-inflammatory cytokines which are released from the stem cells. These cytokines have been found to be involved in improving neurogenesis⁴⁷. Thus, the utilization of stem cells to replace the cells that are lost during the course of epileptogenesis has been considered an effective strategy in the treatment of epilepsy⁴⁸.

In the present investigation, the morphological characteristics of ADMSCs and BMMSCs as fibroblast like cells come in line with the study of Masoud *et al.*⁴⁹. Moreover, flow cytometry analysis revealed that ADMSCs are positive for CD90 and this finding is in conformity with that of Bourin *et al.*⁵⁰. Meanwhile, these cells were negative for CD14 as in the study of Mildmay-White and Khan⁵¹. Similarly, ADMSCs were negative for CD45 and this result is in harmony with that of Bourin *et al.*⁵⁰. Regarding BMMSCs flow cytometry analysis showed that, these cells are positive for CD90 and negative for CD14. These results are in accordance with those of Dominici *et al.*³⁰. Also, BMMSCs were negative for CD45 which is in concert with the report from Chaudhary and Rath⁵².

It has been reported that the rat pilocarpine model resembles human epilepsy⁵³ and its use help investigators to expand knowledge in this field and to develop more effective treatments⁵⁴. The presence of ADMSCs and BMMSCs cells in the brain of the epileptic rats treated with these cells confirmed by the staining of ADMSCs and BMMSCs with PKH26 in the present study. This finding indicated that the transplanted MSCs have survived and accommodated in the site of degradation. This finding converges with the studies Tamura *et al.*⁵⁴ and Abdanipour *et al.*³⁷.

The present study showed that when the pilocarpine-challenged rats were examined by the mean electric shock (MES) test, a significant drop in the MES value was recorded, indicating the

existence of strong seizure, appeared as "quick convulsions". This finding shows parallelism with that of Kitano *et al.*⁵⁵. On the opposite side, a significant rise in the MES values was registered in the other treated groups (epileptic + LEV, epileptic + ADMSCs, epileptic + BMMSCs, epileptic + ADMSCs + LEV and epileptic + BMMSCs + LEV) indicating that LEV, ADMSCs and BMMSCs ameliorated the epileptic impact of pilocarpine and proved the anticonvulsant and antiepileptic effects of ADMSCs and BMMSCs like LEV. The antiepileptic and anticonvulsant activity of LEV was previously reported^{56,57,58}.

The findings of the present work indicated a significant reduction in the brain level of GABA and a significant elevation in the level of brain glutamate, dopamine, bFGF, BDNF, IL-6 and TNF- α , in the rats during acute phase of epilepsy, (24 hours after administration of pilocarpine) in comparison with those of the control counterparts.

The recorded decrease of brain GABA and the increase of brain glutamate levels after pilocarpine administration, agree with the results of the previous studies^{59,60,61,62}. These investigators mentioned that pilocarpine induces a decline in the GABA level and an elevation in the level of glutamate, either in the hippocampus or in the brain cortex. McCormick and Contreas⁶³ reported that seizures can be generated in response to the deterioration between the inhibitory and excitatory balance leading to tonic depolarization or repetitive rhythmic burst discharges. Also, Hasegawa *et al.*⁶⁴ cited that the imbalance of the inhibitory and excitatory neurotransmitters in the brain has been suggested to cause epileptic seizures. It is well known that GABA is the common inhibitory neurotransmitter in the cortical neurons⁶⁵, and glutamate is the main excitatory neurotransmitter in the mammalian central nervous system⁵⁹. Thus, in the present investigation, the drop in the level of brain GABA recorded in the acute phase of epilepsy, may be due to the following: (1) The alterations in the metabolism of GABA, which may have a role in the genesis and propagation of seizure activity as a result of pilocarpine administration⁶⁵, (2) The damage of the GABAergic neurons in the brain cortex of the epileptic animals⁶⁵, (3) Loss of GABA neurons that may represent the epileptic status in acquired epilepsy⁶⁵. Loss of GABA neurons has been observed in the acquired epileptic

models, including kindling, status epilepticus and traumatic brain injury models^{66,67} and (4) A dysfunction of glutamate transporters induced by excess release of glutamate⁶⁴.

The increase in brain glutamate level, obtained in the present work, 24 hours after administration pilocarpine, may be attributed to: (1) The hyperactivity of brain neurons due to the effect of pilocarpine⁵⁹, (2) A possible severe damage in the brain as a consequence of the pilocarpine administration⁶⁴, (3) Activation of glutamate transporters by pilocarpine⁶⁸, and/or (4) An increase in Ca⁺⁺ level which was suggested to increase brain glutamate level and induce status epilepticus⁴³.

In the current research, the level of brain dopamine increased significantly in the rats administered with pilocarpine. This result is in accordance with the previous studies of Cavalheiro *et al.*⁶⁹ and Bozzi and Borrelli⁵, which explained this phenomenon by the abnormal control of dopamine levels or a deviation in the dopamine receptors expression. Pilocarpine displays an unusually elevated dopaminergic neuron drive in the form of an enhancement in the activity of the dopaminergic neuron population⁷⁰. The Ventral Tegmental Area (VTA) of dopaminergic neurons activity is governed by hippocampal VTA loop which involves ventral subicular-nucleus accumbens-ventral pallidal-VTA pathway⁷¹. Therefore, the pathological hyperactivity of the hippocampal formation (including subiculum) can result in increased activity of VTA dopamine neuron. Since the phasic burst firing response of dopaminergic neurons premised on their spontaneous activity⁷², the abnormal high levels of dopaminergic neurons population activity would enable the phasic burst promoter to trigger burst firing in a large number of dopamine neurons, thereby laying the dopaminergic system in a hyper-responsive status in the epileptic brain^{71,73}. Also, the specific receptors of dopamine can modify the neuromodulatory effect of dopamine on brain circuits particularly in the limbic system. The binding potential of D2/D3 receptors has been found to be reduced in the brain of epileptic patients and the decrease in D2 receptor protein expression in the temporal neocortex has been detected in epileptic patients⁷⁴.

The data of the current approach recorded a significant increase in the level of brain bFGF in the pilocarpine administered rats (acute phase

of epilepsy) and this finding is in conformity with the result of Simonato *et al.*⁷⁵ who demonstrated an increased level of bFGF in the hippocampus, neocortex and hypothalamus in the rat kindling model of epilepsy. Also, Gwinn *et al.*⁷⁶ reported a significant rise in the bFGF level in the rat cortex, which remained significantly high up to 72 hours after the electroconvulsive shock. Moreover, Paradiso *et al.*⁷⁷ concluded that the bFGF is highly implicated in the changes of brain functions during the epileptic seizures.

In the current investigation, the level of brain BDNF increased significantly in the pilocarpine-challenged rats. This result is in harmony with that of the previous research works^{78,79}, which mentioned a significant enhancement in BDNF level in several brain regions after administration of a convulsive dose of pilocarpine in animals. It has been proposed that the increased production of BDNF may affect the survival of the neurons and the growth of fiber, thus maintaining the neuronal networks organization in brain area involved in the epileptic activity⁸⁰ and indicating active neosynthesis^{81,82}.

The findings of this work revealed a significant rise in the level of brain IL-6 and TNF- α of the pilocarpine-challenged rats. This finding could be interpreted by the influence of epileptic seizures on the immune system to produce cytokines such as IL-6 and TNF- α ^{83,84}. IL-6 is a multifunctional cytokine that regulates inflammatory responses and other immune reactions. It has been stated that IL-6 level is elevated in the various areas of the rat brain during the epileptic seizures^{85,86}.

Regarding brain TNF- α level, several animal studies reported that after seizures evoked in the rat kindling model of epilepsy, TNF- α levels displayed a significant increase in the brain cortex as well as amygdala and hippocampus. TNF- α has been found to have anticonvulsive effect during the epileptic seizure⁷⁷. Thus, the increased brain level of TNF- α observed in this study in the pilocarpine-challenged rats may be related to its role as an anti-inflammatory mediator and an anticonvulsive factor during the epileptic seizures in the acute phase of epilepsy^{84,87}.

The present data revealed that LEV caused a marked improvement in the changes induced by pilocarpine in rats, since LEV recovered the

behavior of the rats which was observed after pilocarpine administration. Also, a significant increase in GABA level and a significant reduction in glutamate, dopamine, bFGF, BDNF, IL-6 and TNF- α levels were recorded after treatment with LEV. These results may be explained by the antiepileptic and anticonvulsive effects of LEV as reported by previous investigators^{44, 61,88}. Levetiracetam has been reported to be mostly advantageous in repressing seizures in animal models of spontaneous epilepsy, acute seizure and status epilepticus⁸⁹. Potschka *et al.*⁹⁰ cited that LEV is a broad-spectrum antiepileptic drug for the treatment of partial and generalized epilepsy. Also, the anticonvulsive activity of LEV has been mentioned by Lyseng-Williamson⁹¹, where LEV retarded the outset of convulsive activity and lessened the injury of the neurons in the pilocarpine model of epilepsy. Moreover, the anticonvulsant activity of LEV includes the conservation of hippocampal noradrenalin and dopamine levels⁶¹.

The present results showed that the two types of rat MSCs (ADMSCs and BMMSCs) significantly ameliorated all the behavioral signs of pilocarpine administration which were observed in the pilocarpine-challenged rats. The beneficial effectiveness of MSCs in epilepsy has been reported in the previous investigations. Helbokazov *et al.*⁹² stated that the treatment of refractory epilepsy patients with autologous MSCs reduced the seizure frequency. Moreover, it has been suggested that MSCs have the ability to prohibit the development of chronic epilepsy when infused early after the status epilepticus (SE) and alter the disease process after the establishment of the chronic epileptic state²⁵. In parallel with our findings Abdanipour *et al.*³⁷ noticed that the treatment of epileptic rats with BMMSCs yielded improvement in the behavioral experiment as evidenced by the decreased number of seizures in the treated groups. Also, the anticonvulsant effect of ADMSCs has been reported by Tamura *et al.*⁵⁴ who explained this effect by the ability of ADMSCs to block the tonic component of generalized seizures. The present study also revealed that ADMSCs and BMMSCs improved the effect of pilocarpine on the brain biochemical parameters. This is documented by increasing the level of brain GABA and decreasing the level of brain glutamate, dopamine, bFGF, BDNF, IL-6 and TNF- α . It could

be observed that the effect of rat ADMSCs and rat BMMSCs on these biomarkers in the present study is almost similar to that of LEV. These findings indicate that the examined types of MSCs may have antiepileptic and anticonvulsive effects.

D. Souza *et al.*⁹³ commented that the favorable action of MSCs may derive from the induction of local neurogenesis through the secretion of neuronal growth factors, such as (bFGF) and (BDNF). Salem *et al.*⁹⁴ concluded that in an epileptic rat model, treating the rats with BMMSCs ameliorated all the tested biochemical parameters which were markedly perturbed after administration of pilocarpine. The neuroprotective effect of BMMSCs in a cell culture model has been demonstrated when the purified BMMSCs were applied in the early phase after seizure⁹⁵. These authors observed the reduction in neuronal loss and the activation of astrocytes and microglia after treatment with BMMSCs. The research study conducted by Shetty *et al.*²⁷ examined the effect of i.p. infusion of human BMMSCs, one hour after seizure, in epilepsy rat model; these investigators concluded considerable protection of the principal neurons, reduction of gabaergic interneurons, loss normalization of the proinflammatory cytokines' level, enhancement of expression of genes coding anti-inflammatory cytokines in the hippocampus and reduction of myeloperoxidase activity.

ADMSCs proved potent efficacy and efficiency in some clinical applications for both autologous and allogeneic purposes, hence becoming believed as promising tools for substituting, restoration, and regenerating dead or damaged cells⁹⁶. In particular, the seizures induced in transplanted mice have been found to trigger an acute inflammatory condition; altering the pattern of the gene expression of IL1- α , IL-6, iNOS, caspase-1 and IL-4. The immunomodulatory and anti-inflammatory properties of ADMSCs have been documented, providing evidence for utilizing ADMSCs as therapeutic candidate for a wide variety of degenerative diseases. ADMSCs could affect the activity of neurons in the hippocampus by ameliorating the exaggerated and synchronous neuronal firings that yield seizures and or by producing modulatory molecules which could "turn off" the tonic activity of the generalized seizures. One possible reason for the neuroprotective effect of ADMSCs is that the implanted cells act as, a

barrier prohibiting the hyperexcitability of the local circuit and preventing the spread (generalization) of seizures, represented by the lessening in the duration (tonic) and in the post-ictal period. Thus, the anticonvulsant effect of the ADMSCs on acute convulsive seizure may be due to inhibitory factors and immunomodulatory mechanisms⁵⁴. Moreover, ADMSCs could transdifferentiate into neuron-like cells that have multiple neuronal characteristics like synaptic transmission, action potential, neurotrophic factors, and spontaneous postsynaptic current⁹⁷.

Histomorphological investigation of brain tissue section of rat in the epileptic group showed degeneration and atrophy in the neuronal cells of fascia dentata and congestion of blood vessels in the neuronal cells of subiculum of the hippocampus. The striatum in the cerebrum showed focal hemorrhage in the epileptic group. These findings echo those observed by Scorza *et al.*⁴³. Improvement in the histopathological feature of the brain tissue section of rat treated with LEV, ADMSCs, BMMSCs after 3 months has been noticed as represented by the presence of congestion in the cortical blood vessels only. These observations fit those obtained from the previous studies^{15, 21, 98} which documented the capability of MSCs in inducing an increase in the neurogenesis of the hippocampus and a reduction in the neuronal death.

The outcomes of the present study showed a marked difference between the effect of ADMSCs and BMMSCs on the investigated parameters in the brain of the treated rats. This difference may be related to the different tissues from which MSCs are derived as the ADMSCs are derived from the adipose tissue, and the BMMSCs are derived from the bone marrow tissue. This interpretation is concordant with the previous investigators who suggested that MSCs from various sources may have different characteristics^{99, 100, 101}. Also, historic differences between ADMSCs and BMMSCs in the secretome and chemokine receptors expression have been reported by Hsiao *et al.*²⁴ and Elman *et al.*¹⁰². Several secreted molecules such as cytokine (interferon- α), growth factors (basic fibroblast growth factor, hepatocyte growth factor and insulin like growth factor-1), and chemokine (stem cell derived factor-1) have been shown to be more pronounced in ADMSCs than BMMSCs²¹.

The findings of the current investigation revealed that the combined treatment of either ADMSCs or BMMSCs with LEV has a superior effect over the single treatment of each type of the MSCs alone. These results fit similar findings mentioned by Liu *et al.*¹⁰³ and Liu *et al.*¹⁰⁴ who stated that the co-injection of stem cells with the pharmacological therapy can enhance the migratory ability of MSCs to reinforce the damaged sites beside the action of the pharmacological drug.

CONCLUSION

The results revealed that rat ADMSCs and rat BMMSCs may have therapeutic potential as antiepileptic and anticonvulsive candidates in the rat model of acute phase of epilepsy. Noteworthy, the effect of the rat ADMSCs has been shown to be higher than that of the rat BMMSCs in modulation of the relevant biomarkers of epilepsy. Additionally, the combined treatment of either ADMSC or BMMSCs with the anti-epileptic drug (LEV) showed a synergistic effect as it exhibited superior beneficial effect in retrieving the biochemical alterations as well as the histological distortion of brain tissue in comparison with the single use of each type of cells during the acute phase of epilepsy. The outcomes received in this attempt pave the way for establishment of cell-based therapy in combination with the conventional therapy in the treatment of epilepsy.

Limitations of the study

Some crucial questions are needed to be answered by conducting more studies. For example; more studies are needed to confirm therapeutic effect of MSCs in epilepsy models, clarifying how to improve and achieve the long survival time, integration, and long-term functions of transplanted MSCs in the host environment and elucidating the specific differentiation of MSCs and their paracrine therapeutic effects. Also, a longer follow-up time and optimized stem cell administration to accurately test the long-term curing potency of MSCs therapy in epilepsy are demanded. Solving these questions will certainly expedite the realization of the great promise of MSCs-based therapy in epilepsy.

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