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RESEARCH ARTICLE

Insights on the Role of Mesenchymal Stromal Cells Infusion in Enhancing the Inhibitory Network and Synaptic Plasticity in Chronic Epileptic Rats

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Abstract

Objective: Temporal lobe epilepsy (TLE) is characterized by progressive synchronization with high frequencies of drug resistance. Mesenchymal stromal cells (MSCs) could open promising avenues for epileptic therapeutic approaches. The rational of the current research was to realize the therapeutic outcome of bone marrow (BMSCs) and adipose (ADMSCs)-drived MSCs against chronic epileptogenic alterations implicated in pilocarpine-induced TLE. Methods: Adult male *Wister* rats were distributed into five groups: Gp1: non epileptic control group, Gp2: chronic epileptic control group, Gp3: chronic epileptic group treated with BMSCs, Gp4: chronic epileptic group treated with ADMSCs and Gp5: chronic epileptic group treated with carbamazepine (CBZ). Gama-aminobutyric acid (GABA), semaphorin4D (Sema4D) and galanin concentrations were evaluated in brain *via* ELISA. Gene expression level of hippocampal synapsin-I was estimated using sqRT-PCR. Histological explorations of hippocampus and cerebral cortex were carried out. Results: Significant decline in GABA, galanin and Sema4D contents as well as synapsin-I mRNA level were recorded after pilocarpine injection. BMSCs or ADMSCs transplantation or CBZ administration constructed significant upgrade in GABA, galanin and Sema4D concentrations as well as synapsin-I mRNA level. Micrograph of brain tissue sections of epileptic rats showed nucleus pyknosis and neurodegeneration while, the treated groups demonstrated almost intact histological architecture. Conclusion: Based on the forementioned results, this work established the exact mechanisms underlining epileptogenesis and offered a new hope for treatment of focal epilepsies by employment of MSCs which achieved significant neuroprotective activity through modulating epileptic neuronal circuit, secreating neurotrophic molecules and restoring synaptic plasticity.

Keywords: *Chronic epilepsy, mesenchymal stromal cells, neuroprotection, GABA, Sema4D, galanin, synapsin-I.*

Introduction

Temporal lobe epilepsy (TLE) which is the main refractory type of epilepsies affects more than 30% of epileptic people manifested with recurrent seizures [1]. Hippocampal sclerosis (HS) is the main characterized lesion that is recognized in 60-75% of patients with medically untreatable TLE. It is distinguished with severe neuronal defeat and gliosis which caused advance volume loss in the hippocampal region [2]. TLE is discriminated through numerous hippocampal aberrations, concerning of GABAergic interneurons loss, cell defeat, deviant dendritic growth, synaptic disorganization together with hyperexcitability. These features could be

both causal or resultant of the status epilepticus (SE) seizures distinguishing TLE [3]. Moreover, hippocampal injury resulting from SE can lead to a state of chronic epilepsy, characterized by spontaneous recurrent seizures (SRS), cognitive and mood dysfunctions [4]. Currently, the chief therapeutic approaches for TLE are surgery along with anti-epileptic medications that acting *via* reduction of the overall brain firing through hindering sodium ion channels or motivating GABA-ergic channels. However, the curing efficacy is not satisfactory, as well as some additional damages may be occurred [5]. And more, refractory epilepsy, particularly TLE, is resistant to a variety of anti-epileptic drugs [6]. Therefore, improving knowledge on epileptogenesis may assist in creating novel targets for formulating more effective therapies.

Chronic epilepsy as a consequence of status epilepticus has been studied in animal models to analyze different cellular mechanisms causing the subsequent occurrence of spontaneous seizures [7]. Pilocarpine model of epilepsy represents the principle aspects of human TLE, i.e., hippocampal neuronal loss, limbic and secondary generalized seizures as well as SE that continues for several hours [8]. Status epilepticus persuaded by pilocarpine causes a characteristic pattern of neuronal death in the hippocampus; which is followed after an apparent latent period by the development of chronic, recurrent, spontaneous seizures [7]. Pilocarpine-induced epilepsy is achieved principally *via* the activation of M1 muscarinic receptors in the CNS, then the seizures are sustained through N-Methyl-D-aspartate receptor (NMDAR) actuation [9]. This model is considered as an appropriate animal model for studying spontaneous recurrent seizures and related disabilities thus it has the potential to evaluate the therapeutic efficacies of the new treatment options [10].

Mesenchymal stromal cells are multipotent cells that have the aptitude to self-renewal, plastic adherence and trilineage differentiation potential [11]. Additionally, MSCs are capable to survive and retain tissue features in unfavourable microenvironment. MSCs-mediated paracrine activity participates in the initiation of tissue repair [12]. Actually, MSCs based therapies have been applied in the perspective of several neurodegenerative diseases. Neuronal endurance has been demonstrated in animal models of stroke and traumatic brain injury (TBI) infused with MSCs. Moreover, MSCs implantation recovered motor functions of parkinson's disease (PD) induced in animal models as well as in spinal cord injury (SCI) model with detectable remyelination and neural circuitry reconnection [13]. Gathered evidences attribute the regenerative probability of MSCs to their capability to secrete numerous biomolecules as well as trophic factors, namely neurotrophic growth factors, chemokines, cytokines, extracellular matrix proteins and extracellular vesicles [14]. Pre-clinical and clinical observations illustrated that these molecules can directly motivate the recruitment proliferation, and differentiation of the endogenous stem cells [15]. Mesenchymal stromal cells could harmonize local mechanisms involving apoptosis, scarring, oxidation and revascularization, as well as modulation of immune and inflammatory responses [16], thus lessening tissue deterioration. In particular, robust neuroprotective effects of MSCs transplantation were manifested in rodent models of epilepsy [17]. It is possible that the anti-epileptic response of MSCs are cased by recuperating the balance between inhibitory and excitatory signaling throughout transplanting cells that develop GABAergic neurons in addition to neuroprotective astrocytes [18]. Moreover, MSCs infusion has been found to reduce GABA-ergic interneurons loss, inhibit myeloperoxidase activity, down-regulate NMDAR subunit expression, blunt glutamate-provoked calcium influxes and up-grad expression of anti-inflammatory cytokines encoding genes in the hippocampus [19].

The present attempt was mainly focused on gaining a better perception of the pathomechanism of a state of chronic epilepsy induced by pilocarpine inoculation in rats and define the feasible neuroprotective behavior of mesenchymal stromal cells agaist TLE through tracing the mechanisms by which they act.

Materials and Methods

Mesenchymal Stromal Cells

Derivation and Culturing of Mesenchymal Stromal Cells

Bone Marrow Mesenchymal Stromal Cells (BMSCs)

Bone marrow mesenchymal stromal cells (BMSCs) were segregated from the tibias and femurs bone marrow of two month old male *Wister* rats. Concisely, rodents were sacrificed *via* decapitation and swiped with 70% ethanol pursued by sterile dissection. After the sacrifice of the rodents, the tibias and femurs were erased and cleaned from connective tissues. Epiphysis bones were cut, and bone marrow was flushed out, then suspended in Dulbecco's modified Eagle's medium (DMEM) [Biowest the serum specialist, 422 NW Business Park Lane, Riverside, MO 64150, USA] supplemented with heparin to avoid clotting. In order to harvest the mononuclear cells, marrow in DMEM was centrifuged at 1800xg for 20 mins. The cell pellet was re-suspended with complete DMEM culture medium enhanced with 10% fetal bovine serum (FBS) [Biowest, USA] as well as 1% penicillin-streptomycin [Biowest, USA] [20].

Suspended bone marrow cells were cultured in 25 cm^2 flasks, then were incubated with complete culture medium at 37 °C in 5% humidified CO_2 incubator for fourteen days (primary culture) till colonies formation. Medium was changed every three days for eliminating non-adherent cells. Culture flasks were observed continuously *via* inverted microscope [21].

After 70-80% confluence, the cultures were washed twice with phosphate buffer saline (PBS) [Biowest, USA], then the cells were trypsinized with 0.25% trypsin in 1mM ethylene diamine tetraacetic acid (EDTA) solution [Biowest, USA] for 5 mins at 37°C. This unremitting culturing steps outcomed removal of non-adherent (non-BMSCs) cells and gradual purification of the BMSCs. These cells were called passage 1 that were harvested by trypsin-EDTA solution and passaged to subculture again. Once the adherent cells in passage 2 reached 70–80% confluence, passage 3 BMSCs were employed [22].

Adipose Mesenchymal Stromal Cells (ADMSCs)

For adipose tissue harvesting, three month aged adult male *Wister* rats, weighing 250 to 300 g were sacrificed. Fats were surgically gethered from rat omentum and gently incised with strillized scissors into small pieces that washed delicately for 3-4 times with equal volume of PBS. Then, they were incubated for 60 mins at 37˚C in shaker incubator with a solution of 0.2% collagenase type I (Sigma-Aldrich, USA) in PBS and centrifuged at 1000 xg for 10 mins.

After that, 10% FBS was added in order to inactivate collagenase enzyme, then the mixture was transfered into 50 mL falcon tube that was centrifuged at 1000 xg for 10 mins at room temperature with the purpose of separating the stromal vascular fraction (SVF) from the oil and remaining fat lobules. Floating adipocytes, lipids as well as liquids were aspirated. For lysing red blood cells, SVF pellet was incubated with 160 mM NH_4Cl at room temperature for 10 mins. Additionally, the mixture was centrifuged at 400 xg for 10 mins at room temperature then applied for a ficoll gradient (Sigma-Aldrich, USA) to purify the mononucleated cells. The mixture was centrifuged at 1000xg for 30 mins at room temperature and the mononucleated cells were washed with PBS for two times and centrifuged at 400xg for 10 mins between each wash. The pellet was re-suspended in PBS, filtered and centrifuged at 400xg for 10 mins. SVF cells were filtered, centrifuged and re-suspended in DMEM with 10% FBS and 1% penicillin-streptomycin solution [23] then cultured in 25 cm^2 flasks. The non-adherent cells were removed by PBS washing after 24 hrs of culturing. The cells were preserved at sub-confluent levels in a 37˚C incubator with 5% CO_2 as well as passaged with trypsin / EDTA when necessitated as indicated previously with BMSCs. Then adherent adipose stromal cells were seeded in 25 cm^2 culture flasks with complete culture medium [24].

Mesenchymal Stromal Cells Description

Morphological Appearance of MSCs

MSCs were identified by their spindle-like shape under the inverted microscope [25].

Gene Expression of MSCs Surface Markers

Mesenchymal stromal cells were identified by detection of gene expression of CD90, CD105,

CD14 and CD45 (surface signals for MSCs) by conventional polymerase chain reaction (PCR) to ensure the phenotype of MSCs.

Detection of CD90, CD105, CD14 and CD45 Gene Expressions *via* Conventional Polymerase Chain Reaction

RNA Extraction

Total RNA was extracted from BMSCs and ADMSCs by RNA-Spin™ total RNA extraction kit for purification of total RNA from animal cells/tissues (Intron Biotechnology Co., kyeonggi-do, Korea, Cat.No.17211) according to the manufacturer's directions.

Reverse Transcription

The extracted RNA was reversely transcripted using the Thermo Scientific Revert Aid First Strand cDNA Synthesis kit (Thermo Fisher Scientific Inc., USA, Cat. No. K1622) according to the manufacturer's instructions.

Conventional PCR Detection

The semi-quantitative RT-PCR was carried out using the first strand cDNA as templates with a pair of specific primers in 50 µL reaction volume using 2x master mix solution (1-Taq™) (Intron, Korea) according to the manufacturer's protocol.
Oligonucleotide primers sequences of studied genes: CD90: Forward 5′ GTGAACCAGAGCCTTCGTCT 3′; Reverse 5′ GGTGGTGAAGTTGGACAGGT 3′ [26]; CD105: Forward 5′ CACTAGCCAGGTCTCGAAGG 3′; Reverse 5′ CTGAGGACCAGAAGCACCTC 3′ [27]; CD45: Forward 5′ACCAGGGGTTGAAAAGTTTCAG 3′; Reverse 5′ GGGATTCC-AGGTAATTACTCC 3′ [28]*;* CD14: Forward 5′ GTGTGAGTGGTAGCCAGCAA 3′; Reverse 5′ TGCGCAGCGCTAAAACTTG 3′ [29]*;* ß-actin: Forward 5′AGTGTGACGTTGACATCCGTAA 3′; Reverse 5′GGACAGTGAGGCCAGG-ATAGA 3′ [30].

The reaction mixture of RT-PCR consisted of twenty five µL of 2X PCR master mix solution (Intron, Korea), one µL of the corresponding forward primer with conc. of 10 pmol/µL (Invitrogen, USA), one µL of the corresponding reverse primer with conc. of 10 pmol/µL (Invitrogen, USA), two µL of cDNA and twenty one µL of RNase free water with a total reaction volume fifty µL. The mixture was blended well *via* pipetting then it was spinet down through brief centrifugation. The PCR was accomplished using thirty-five cycles with initial denaturation step at 94°C for 2 mins. Each cycle involved denaturation at 94°C for 20 s, annealing at 57 °C for CD 45, 55 °C for CD 90 and at 60 °C for CD105 as well as CD14 for 20 s, extension at 72°C for 1 min followed by 5 mins of final extension at 72°C at the end of the last cycle. The quantitative values of RT-PCR for these genes were normalized on the bases of ß-actin expression. The PCR products were separated *via* electrophoresis through 2.0% agarose gel, stained with ethidium bromide, and photographed by gel documentation system.

Staining of MSCs with PKH26 dye for Verification of Accomodation

The third passage of mesenchymal stromal cells were gathered and marked with PKH26 dye by using the PKH26 Fluorescent Cell Linker kits (Sigma-Alderich, USA) according to the manufacturer's protocol **[31]** to illustrate their homing in the chronic epileptic rat brain tissues.

In vivo Experimentation

Drugs and Chemicals

Pilocarpinium nitrate was acquired from MERCK CO., 2000 Galloping Hill Road Kenilworth, NJ 07033, USA. Methylscopolamine bromide was supplied from Sigma-Aldrich, USA. Calmepam® (Bromazepam) tablets, 1.5 mg were obtained from GlaxoSmithKline Co., Egypt. Tegretol® (Carbamazepine) tablets, 200 mg were purchased from Novartis Pharma Co., Egypt under license from Novartis Pharma AG., Basle, Switzerland. All other chemicals used for analysis met the quality criteria in accordance with international standards.

Animals

Animals were cared for according to the guidelines for animal experiments which were approved by the Ethical Committee of Medical Research of the National Research Centre, Giza, Egypt (Ethical code: 14-018) that conform to the recommendations of the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Seventy-five adult male *albino* rats of *Wister* strain (200-230 g) were procured from the Animal House Colony of the National Research Centre, Giza, Egypt and

acclimatized in well ventillated environment where the temperature (25±1°C), humidity (55%) and 12 hrs dark/light cycles for one week before initiating the experiments. Rats were housed with free access to standard laboratory diet and water. All experiments were carried out according to the guidlines of Committee for the Purpose of Contral and Safety of Experimental Animals (CPCSEA).

Model Generation and Cell Implantation

Animals Grouping

The animals in this study (75 adult male *albino* rats of *Wister* strain) weighting (200-230g) were randomized into 5 equal groups according to different treatment schedule as illustrated in Table 1. Rats were noticed for 30 mins and during this period seizures were registered using Racine scale [38] which consisted of 6 stages as follows*:* 1) immobility and rigid posture; 2) mouth movements, head nodding, and repetitive movements; 3) forelimb clonus; 4) rearing and falling; 5) jumping; and 6) severe tonic–clonic seizures. Only the rats exhibiting continuous convulsive SE at stages (1-6) of Racine's scale were included for subsequent experimental manipulations.

Table 1: Animals grouping and treatment schedule

S.NO	Groups	Treatments
1	Non epileptic control group (control group)	The rats were injected with all drugs taken by chronic epileptic control group except pilocarpine that was replaced with equivalent volume of saline (Nacl 0.9 %, by intraperitoneal injection).
2	Chronic epileptic control group (epileptic group)	The rats were injected intraperitoneally with methylscopolamine (1mg/kg b.wt.) [32] 30 mins prior to intraperitoneal pilocarpine injection (380 mg/kg b.wt.) [33] to restrict its peripheral adverse events. After that, all animals received two doses of diazepam separated by one hour (each 4 mg/kg b. wt., intragastrically) [34] 30 mins after seizure onset to end status epilepticus and to optimize the interval of seizure activity. These rats were sacrificed after two month from final administration [35] till the occurrence of spontaneous recurrent seizure (SRS).
3	Chronic epileptic group treated with BMSCs (BMSCs group)	The rats were treated as in group two, after that they were infused with undifferentiated bone marrow mesenchymal stromal cells (4 x 10^6 cells/rat) intravenously [36] and left for four month before being sacrificed.
4	Chronic epileptic group treated with ADMSCs (ADMSCs group)	The rats were treated as in group two, after that they were infused with undifferentiated adipose mesenchymal stromal cells ($4x10^6$ cells/ rat) intravenously and left for four month before being sacrificed.
5	Chronic epileptic group treated with carbamazepine (CBZ group)	The rats were treated as in group two, after that they were received carbamazepine as a referance drug for epilepsy in a daily dose of 120 mg/kg b.wt. (40 mg/kg b.wt., intragastrically, three times/day) [10, 37] for four month before being sacrificed.

Mesenchymal Stromal Cells Transplantation

In short, animals were put into a specialized rodent holder/ restrainer then the MSCs with known count were re-suspended into 100 µL DMEM for each animal. After that, they were slowly injected (3 mins.) into the caudal vein with a needle. The needle was kept in the tail vein for other 3mins. to avoid regurgitation then withdrawn.

Dissection and Brain Tissue Preparation

At the end of the experimental period, rats were sacrificed by cervical dislocation under pentobarbital sodium anesthesia after one night of food deprivation. The whole brain of four rats was promptly excised, immediately frozen in liquid nitrogen and stored at -80º C. Frozen brain was then dissected and hippocampus was excised to be immediately frozen in liquid nitrogen, then stored at -80º C till extraction of RNA for molecular genetic analysis.

On the other hand, the whole brain of each of the remaining rats in each group (6 rats) was dissected, thoroughly washed with ice-cold isotonic saline, blotted dry and sagitally partitioned into two portions. The first portion was fixed in 10% neutral buffered formalin solution to be used for histological studies. While, the second portion of each brain was weighed and homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose, pH 7.4 [39].

The homogenates were centrifuged at 1800xg for 10 min in cooling centrifuge at 4 ˚C. The supernatants (10%) were used for the biochemical evaluations (GABA, Sema4D and galanin). Total protein content was also measured by lowry method to express the concentration of different biochemical parameters as mg^{-1} protein [40].

Biochemical Analyses

Brain GABA content was estimated by ELISA using rat gamma-aminobutyric acid assay kit acquired from Glory Science Co. Ltd, 2400 Veterans Blvd., Suite 16 -101, Del Rio, TX 78840, USA, (Catalog. No: 30313) according to the standard protocol provided with the assay kit. While, Sema4D and galanin concentrations in the brain were assayed using rat ELISA kits obtained from WKEA MED SUPPLIES CORP, Chenguang Gardon, Qianjin Street, Changchun, China, (Catalog. No: WAR-714 and WAR-713 respectively) in accordance with the manufacturer's mannuals. Lastly, quantitative detection of total protein was carried out by chemical method according to that described by [41].

Molecular Investigation

Expression of synapsin-I gene and ß-actin as a house keeping gene were analyzed in this study by using semi-quantitative conventional PCR reaction. The primer sequences designed to amplify these genes are as follows: ß-actin: Forward 5′AGTGTGACGTTGACATCCGTAA3′; Reverse 5′GGACAGTGAGGCCAGGATAGA 3′ [30]*;* Synapsin-I: Forward 5′-GCCTTCAGCATGGCACGTA-3′; Reverse 5′-CAGCATACTGCAGCCCAATG-3′ [42].

RNA Extraction

Total RNA was extracted from rat brain using RNA-Spin™ Total RNA Extraction kit for purification of total RNA from animal cells/tissues (Intron Biotechnology Co., kyeonggi-do, Korea, Cat.No.17211) according to the manufacturer's protocol.

Conventional PCR

RT-PCR was carried out using the Quant One-step RT-PCR Kit (procured from Tiangen Biotech. CO., China, Cat. No. KR113) under the guidance of the manufacturer.

The reaction mixture composed of: five µL of 10X RT-PCR Buffer, two µL of Super pure dNTP Mixture, ten µL of 5X RT-PCR Enhancer, half µL of RNasin (40U/µL), two and half µL of Hotmaster *Taq* Polymerase (2.5U/µL), half µL of Quant RTase, three µL of Forward Primer (10µM) (Invitrogen, USA), three µL of Reverse Primer (10µM) (Invitrogen, USA), two µL of Template RNA and twenty one and half µL of RNase-free ddH_2O for total volume fifty µL.

Thermal cycler conditions were adjusted as follows: reverse transcription at 50°C for 30mins then initial denaturation step at 94°C for 2 mins followed by 40 cycles each cycle consisting of denaturation step at 94°C for 1min, annealing at 56 °C for ß-actin and at 52°C for synapsin-I for 1min, extension at 72°C for 2 mins followed by final extension at 72°C for 7 mins. The PCR products were analyzed using 2% agarose gel electrophoresis and band intensities were measured with Gene Tools analysis software (SynGen, U.K.).

Histopathological Examination

After fixation of brain tissue from each group for 24 hrs, washing was carried out under the running tap water and dehydration with 50%, 70%, 90%, 100% ethanol was done. Then, the specimens were cleared in xylene and finally embedded in paraffin bee wax at 56 degree in hot air oven for twenty four hours. Paraffin blocks of tissues were cut into 4 microns thick slices by using rotary

microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for investigation through the light electric microscope [43].

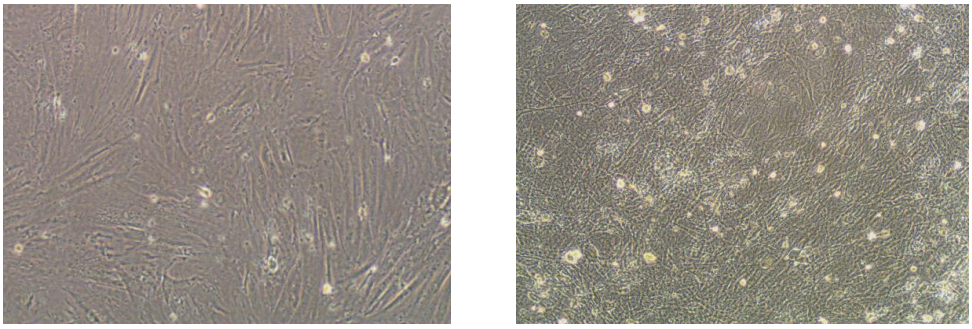
Statistics

In the current study, all results were expressed as Mean ± S.E of the mean. Data were analyzed *via* one way analysis of variance (ANOVA) which was performed using the Statistical Package for the Social Science (SPSS) program, version 25 followed by least significant difference (LSD) for comparing significance between groups [44]. Difference was considered significant when P value was <0.05.

Results

Mesenchymal Stromal Cells Morphology

BMSCs and ADMSCs are recognized through their adhesiveness to the wall of plastic culture flasks and morphological spindle-shape under the inverted microscope as illusterated in Fig. 1 (a,b) respectively.

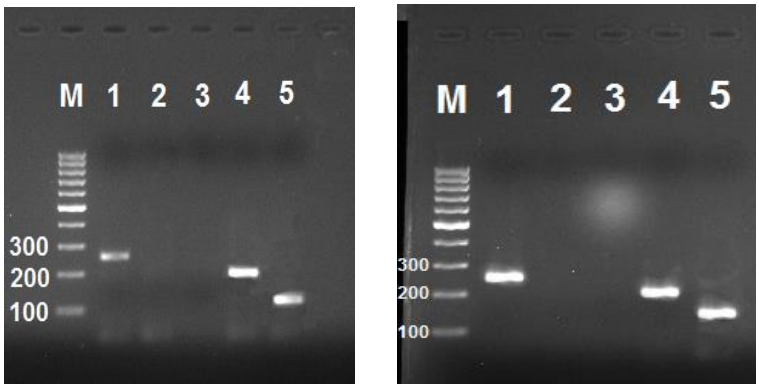


(a) **(b)**

Fig. 1: Morphological appearance of (a) BMSCs and (b) ADMSCs under the inverted microscope

Mesenchymal Stromal Cells Surface Markers

The results of the gene expression of MSCs surface markers obtained in **Fig. 2 (a, b)** revealed that both BMSCs and ADMSCs were positive for CD90 gene (band at 201 bp) and CD105 gene (band at 165 bp) respectively. As well as no expression for CD14 and CD45 genes were observed in the investigated MSCs cells.

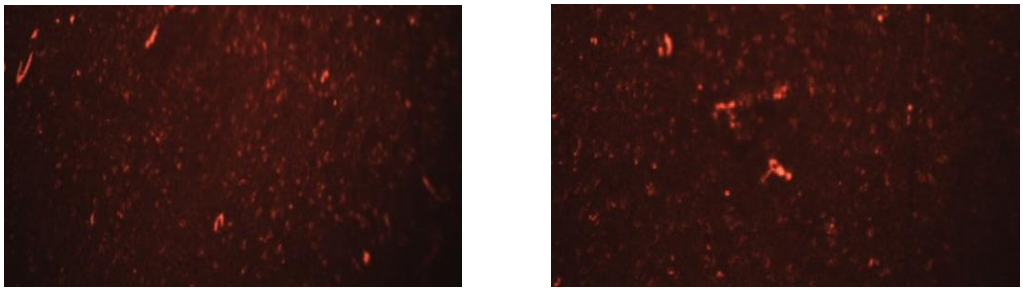


(a) **(b)**

Fig. 2: Agarose gel electrophoresis illustrated β-actin, CD14, CD45, CD90 and CD105 genes expression for BMSCs (a) and ADMSCs (b). Samples: M: DNA ladder (100 bp); Lane (1) β-actin gene expression; Lane (2) CD14 gene expression; Lane (3) CD45 gene expression; Lane (4) CD90 gene expression and Lane (5) CD105 gene expression.

Brain Homing Manifestation using PKH26 Dye

Examination of brain sections of MSCs infused groups using fluorescence microscope displayed that bone marrow **(Fig.3 (a))** and adipose **(Fig.3 (b))** mesenchymal stromal cells stained with PKH26 dye were discovered in different brain areas of BMSCs and ADMSCs groups



(a) **(b)**

Fig. 3: The fluorescence microscope photograph showing homing of PKH26 stained BMSCs (a) and ADMSCs (b) in the brain tissues.

making sure of MSCs homing in damaged epileptic rats brain.

In vivo Experiment Findings

Spontaneous Recurrent Seizures Induction

Ninety percent of pilocarpine administered rats manifested different signs of seizures progressed to SE which were scored using Racine scale started with rigid posture, mouth movement, bilateral limbs convulsion, rearing and falling around thirty min. into a generalized convulsive stage lasting about 30 s to 1 min, one to several times per day. At the end, diazepam administration constricts behavioural seizures in most rats.

Biochemical Outcomes

The data depicted in **Table (2)** presented BMSCs, ADMSCs and carbamazebine deliverance effect on brain GABA, Sema4D and galanin levels in the studied groups.

Table 2: Effect of treatment with BMSCs, ADMSCs and carbamazepine on brain GABA, Sema4D and galanin levels in chronic epileptic rat model. Data were expressed as Mean ± S.E of 6 rats/ group.

Parameters / Groups	GABA (µmol/mg.protein) Mean ± S.E	Sema4D (ng/mg.protein) Mean ± S.E	Galanin (µg/mg.protein) Mean ± S.E
Control group	88.20 ± 1.27	20.14 ± 0.39	50 ± 1.07
Epileptic group	57 ± 2.5 [a] (-35.37%)	9.13 ± 0.37 [a] (-54.67%)	40± 0.75 [a] (-20%)
BMSCs group	71.3 ± 1.43 [b] (25.09%)	13.80 ± 0.39 [b] (51.15%)	44.7 ± 1.21 [b] (11.75%)
ADMSCs group	74 ± 2.23 [b] (29.82%)	14.34 ± 0.56 [b] (57.06%)	4 5 ± 0.71 [b] (12.50%)
CBZ group	65.7 ± 2.67 [b,d] (15.26%)	12.17 ± 0.31 [b,c,d] (33.30%)	43.85 ± 1.47 [b] (9.62%)

a: Significant change at P<0.05 in comparison with the control group.
b: Significant change at P<0.05 in comparison with the epileptic group.
c: Significant change at P<0.05 in comparison with BMSCs group.
d: Significant change at P<0.05 in comparison with ADMSCs group.
(%): percent of difference with respect to corresponding control value.

The epileptic group exhibited significant depltion in brain GABA (-35.37%), Sema4D (-54.67%) and galanin (-20%) contents compared to the control group. Diversely, significant enhancement in brain GABA, Sema4D and galanin levels were detected in the epileptic groups treated with BMSCs (25.09 % for GABA, 51.15% for Sema 4D or 11.75 % for galanin), ADMSCs (29.82% for GABA, 57.06 % for Sema4D or 12.50 % for

galanin), and carbamazepine (15.26 % for GABA, 33.30 % for Sema4D or 9.62 % for galanin), versus the epileptic group.

Interestingly, the epileptic group treated with carbamazepine displayed a significant blunting in brain Sema4D content as compared to the epileptic group treated with BMSCs. Furthermore, the epileptic group treated with carbamazepine elucidated a significant decline in brain GABA and Sema4D levels versus epileptic group treated with ADMSCs.

Molecular Output

Table 3 and Fig. 4 illustrate the therapeutic outcome of BMSCs, ADMSCs and carbamazepine on hippocampal mRNA level of synapsin-I in chronic epileptic rat model.

Pilocarpine administration produced significant down-regulation in hippocampal gene expression level of synapsin-I (-62.55%) versus that in the control group. On the contrary, implantation of BMSCs, ADMSCs or administration of carbamazepine to epileptic group caused significant up-regulation in synapsin-I mRNA level by (128.57%), (112.09%) and (90.11%) respectively when matched up with the epileptic group.

Interestingly, supplying the epileptic group with carbamazepine clarified significant down-regulation of hippocampal gene expression level for synapsin-I in respect with BMSCs or ADMSCs groups.

Table 3: Effect of treatment with BMSCs, ADMSCs and carbamazepine on hippocampal gene expression level of synapsin-I in chronic epileptic rat model. Data were expressed as Mean ± S.E of 4 rats/group

Parameter / Groups	Relative expression of synapsin-I gene (synapsin-I /β-actin)
Control group	2.43 ± 0.04
Epileptic group	0.91 ± 0.05 [a] (-62.55%)
BMSCs group	2.08 ± 0.08 [b] (128.57%)
ADMSCs group	1.93 ± 0.07 [b] (112.09%)
CBZ group	1.73 ± 0.02 [b,c,d] (90.11%)

a: Significant change at P<0.05 in comparison with the control group.
b: Significant change at P<0.05 in comparison with the epileptic group.
c: Significant change at P<0.05 in comparison with BMSCs group.
d: Significant change at P<0.05 in comparison with ADMSCs group.
(%): percent of difference with respect to corresponding control value.

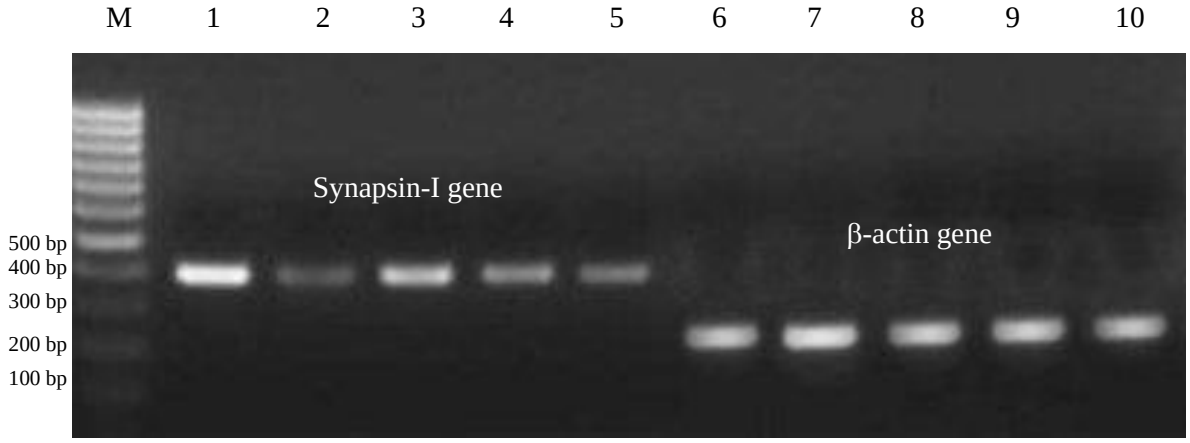


Fig. 4: Expression of synapsin-I as well as β-actin genes in hippocampus tissues collected from rats in the different studied groups. M: represents DNA ladder. Lanes 1&6: represent control group. Lanes 2&7: represent epileptic group. Lanes 3&8: represent BMSCs group. Lanes 4&9: represent ADMSCs group. Lanes 5&10: represent CBZ group.

Histopathological Observations

Microscopic examination of the transverse section through the brain of rats in the control group showed intact neuronal histological structure of the cerebral cortex (Fig.5.a), hippocampal subiculum (Fig.5.b) and fascia dentata as well as hilus (Fig.5.c) regions. On the other side, microscopic investigation of the transverse section through the brain of rats in the epileptic group revealed nucleus pyknosis and degeneration in the most neurons of cerebral cortex (Fig.5.d), hippocampal subiculum (Fig.5.e), fascia dentata and hilus (Fig.5.f) areas. Meanwhile, implantation of BMSCs after pilocarpine administration could maintain the architectural integrity of most neurons of cerebral cortex (Fig.5.g), while, it could restore the normal morphological structure of neurons in subiculum (Fig.5.h), as well as in fascia dentata and hilus (Fig.5.i) in the hippocampus. Photomicrography of the transverse section through the brain of rats in the epileptic group treated with ADMSCs represented nucleus pyknosis and degeneration in some neurons of cerebral cortex (Fig.5.j) while, hippocampal neurons appeared with normal structural organization in subiculum (Fig.5.k), fascia dentata and hilus (Fig.5.l). Microscopic follow up and photodocumentation of the transverse section through the brain of rats in the carbamazepine group indicated the presence of nucleus pyknosis along with degeneration in most neurons of cerebral cortex (Fig.5.m), while, the micrograph of subiculum of hippocampus demonstrated normal histological architecture of the neurons (Fig.5.n). However, photomicrograph of hippocampal fascia dentata and hilus areas clarified nucleus pyknosis and degeneration in some neurons (Fig.5.o).

Discussion

The objective of the present investigation was to examine bone marrow mesenchymal stromal cells or adipose mesenchymal stromal cells performance to alleviate epileptogenesis in temporal lobe epilepsy rodent model induced *via* pilocarpine systemic administration.

Neurological destructions are frequently irreversible because of limited regeneration in the CNS along with the challenge in crossing drugs through the blood brain barrier (BBB) [45]. Consequently, based on the regenerative capacity of MSCs in addition to their crossing ability throughout BBB, their implantationhave been inspected in basic research and pre-clinical explorations illustrating immense prospects in curing various neurological disorders [46].

Our results fit with those obtained by Jezierska-Woźniak et al. [47] and Mohi El-Din et al. [48] who illustrated that the isolated MSCs adhered to the wall of the plastic flasks and extensively proliferated with fibroblast-like morphology.

Bone marrow mesenchymal stromal cells isolated in the current study were positive for CD90 plus CD105 while, were negative for CD14 as well as CD45. These findings are in harmony with those of Hlebokazov et al. [49] who showed that BMSCs are immunophenotyped through flow cytometry as $CD90^{+}$, $CD105^{+}$, $CD45^{-}$ and $CD34^{-}$ cells. Moreover, our results revealed that the segregated ADMSCs are positive for CD90 and CD105 genes but are negative for CD45 and CD14 genes. These observations comes in line with those of Vyas et al. [13] who clarified that fluorescence activated cell sorting ADMSCs verifies $CD90^{+}$ $CD105^{+}$ $CD45^{-}$, $CD34^{-}$ cells in addition to Schwerk et al. [50] who disclosed the majority of ADMSCs are negative for the markers of CD11b, CD14, CD19, CD34 and CD45 nevertheless almost all cells expresse CD29, CD44, CD90 and CD105 surface markers.

The data proposed in this research indicated that the intravenously implanted MSCs are capable of transferring to brain damage site. The homing nature of MSCs is due to their wider expression of homing molecules [51]. This observation is supported by Matsuda et al. [52] who stated that intravenously injected MSCs move and incorporate like neural stem cells. Mesenchymal stomal cells could simply access the injured tissue to achieve their therapeutic effect *via* discharging trophic factors. Non-human primates implantation experiments have elucidated that MSCs have the ability to spread through several tissues subsequent to intravenous administration where it preferentially home to the site of injury, for sustaining functional recovery [53]. The trafficking of MSCs is chiefly consists of activation, adhesion as well as transendothelial migration. In response to injury the immune signals, homing receptors as well as chemokines are prompted and released that therefore motivates MSCs. Then, MSCs are transferred to the peripheral

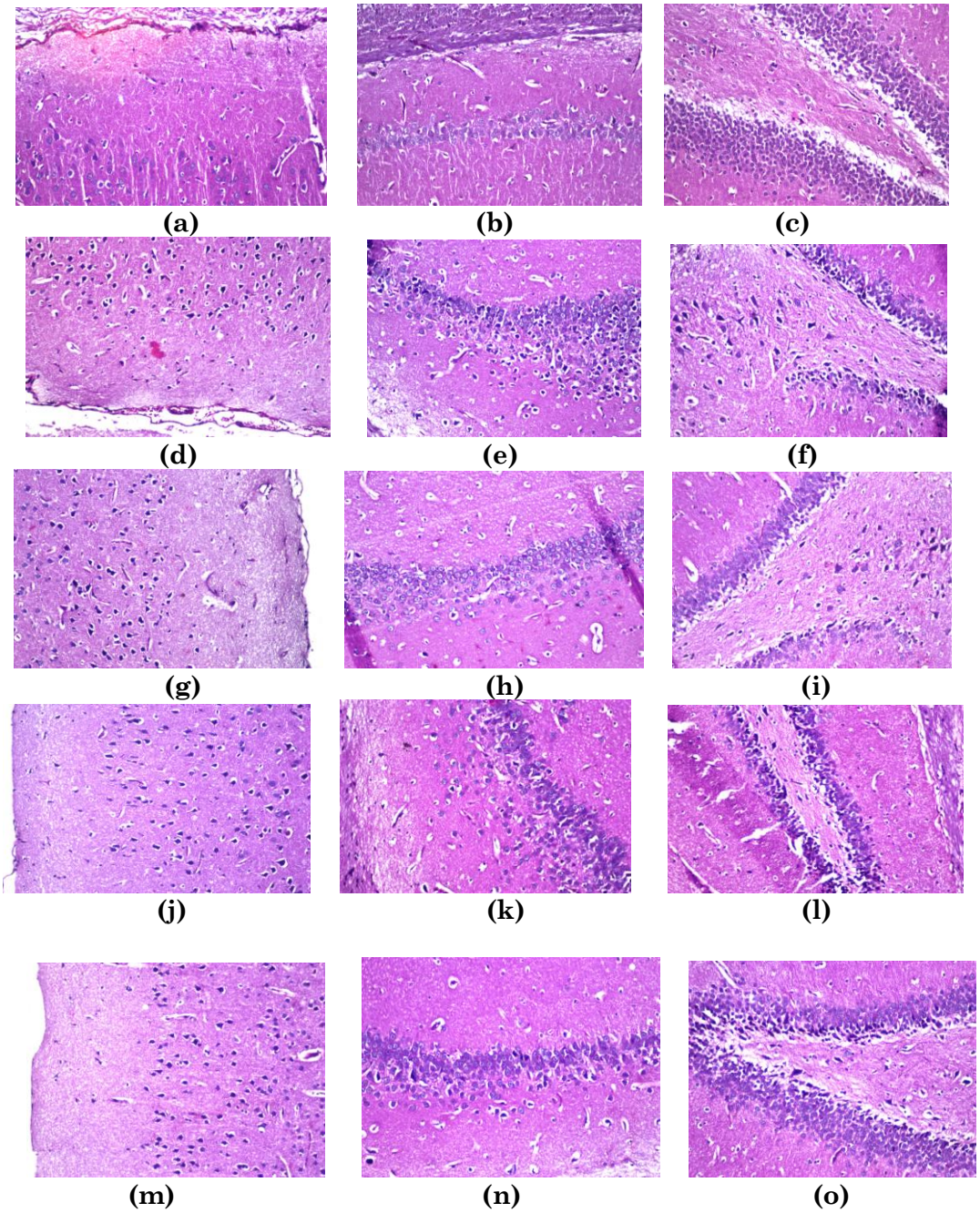


Fig. 5: Photomicrograph of brain tissue section of rat (H&EX40): (a) illustrating normal histological structure of the neurons in cerebral cortex in the control group. (b) showing normal histological structure of neurons in the subiculum of hippocampus in the control group (c): clarifying normal histological structure of neurons in the fascia dentata and hilus of hippocampus in the control group. (d): manifesting nucleus pyknosis and degeneration in most neurons of cerebral cortex in the epileptic group. (e): un-covering nucleus pyknosis and degeneration in most neurons of subiculum in hippocampus in the epileptic group. (f): demonstrating nucleus pyknosis and degeneration in most neurons of fascia dentata and hilus in hippocampus in the epileptic group. (g): showing nucleus pyknosis and degeneration in most neurons of cerebral cortex in epileptic group treated with BMSCs. (h): splashing normal histological structure of neurons in subiculum of hippocampus in epileptic group treated with BMSCs. (i): representing normal histological structure of neurons of fascia dentata and hilus in hippocampus in epileptic group treated with BMSCs. (j): illustrating nucleus pyknosis and degeneration in some neurons of cerebral cortex in epileptic group treated with ADMSCs. (k): manifesting normal histological structure of neurons in subiculum of hippocampus in epileptic group treated with ADMSCs. (l): clarifying normal histological structure of neurons of fascia dentata and hilus in hippocampus in epileptic group treated with ADMSCs. (m): showing nucleus pyknosis and degeneration in most neurons of cerebral cortex in epileptic group treated with carbamazepine. (n): splashing normal histological structure of neurons in subiculum of hippocampus in epileptic group treated with carbamazepine. (o): un-covering nucleus pyknosis and degeneration in some neurons of fascia dentata and hilus in hippocampus in epileptic group treated with carbamazepine.

blood circulation where the consequential adhesion step is frequently realized by the specific interaction between many chemokines and homing receptors, involving stromal cell-derived factor-1 (SDF-1), CXC chemokine receptor (CXCR), hepatocyte growth factor (HGF), hyaluronic acid (HA), CD44, monocyte chemoattractant proteins (MCPs) and CC chemokine receptor (CCR) interaction [54].

The data accomplished from our study utilizing pilocarpine epileptic model showed significant blunting in brain GABA content. These outcomes fit those obtained by Salem et al. [55] who elucidated that pilocarpine infusion fundamentally diminishs hippocampal GABA level as compared with the control counterparts. Honda et al. [56] explained that pilocarpine decreases GABA discharge by depolarizing the membrane and through a pre-synaptic activity. On the opposite side, Khongsombat et al. [57] reported that pilocarpine results in a short-term (2-3 h) elevation of extracellular hippocampal GABA concentration while no progressions are seen at 24 h. Besides, other studies uncovered transient or inconsequential height in hippocampal GABA expression all through pilocarpine-incited SE [58].

Loss of GABAergic restraint could amplify excitatory postsynaptic potentials along with synchronizing burst discharges, so in this way epileptogenesis was induced [59]. The GABAergic impairment mechanisms may include decrease GABA release [60], alteration of GABA receptors [61], imperfection of GABA synthesis [62] as well as GABAergic neuronal loss [63]. One eventual epileptogenic mechanism in TLE is defeat of GABAergic interneurons as well as inhibitory synapses in the dentate gyrus [64].

Epilepsy amplifies pro-inflammatory cytokines quantities including IL-1β as well as TNF-α in the parietal cortex, hippocampus and amygdala. These cytokines up-managed GABA transporter subtypes GAT-1 and GAT-3 expression levels, as such they diminish GABA content adding to upgraded neuronal excitability in those brain areas [65].

In perspective of our records, BMSCs or ADMSCs treatment in chronic epileptic rats caused significant rise in brain GABA concentration. These data bolstered with Salem et al. [55] who showed that implantation of BMSCs *via* intracranial or intravenous courses fundamentally advances hippocampal GABA content when contrasted with the epileptic group. As well, Mohammed et al. [64] demonstrated that MSCs improves pentylenetetrazol (PTZ)-prompted epileptogenesis by means of seizure control, anti-oxidant and GABA level upgrade in trial model of epilepsy. Additionally, Shetty [66] registered results of intraperitoneal infusion of human BMSCs an hour after SE that accomplishs significant neuroprotection and lessen GABAergic interneurons misfortune in the hippocampus.

Mesenchymal stromal cells relocation and homing abilities towards deteriorated territories would advance neuronal networks and synaptic transmission in epileptic rat model [67]. They could specifically enhance hippocampal GABAergic pre-synapses, support glial-subordinate neuronal persist and trigger amplification of GABAergic transmission in hippocampal cultures [68]. In addition, Long et al. [69] observed that protein levels and additionally immunohistochemical consequences of hippocampal GABA, glutamic acid decarboxylase 67 (GAD67) and GABA-B receptor (GABA-BR) are altogether expanded after MSCs implantation in a chronic cerebral hypo-perfusion rodent model comparative to saline administered rodents. Also, Long et al. [70] illustrated the MSCs capacity to discharge GABA neurotransmitter, vascular endothelial growth factor (VEGF), brain derived neurotropic factor (BDNF) and nerve growth factor (NGF), that can enhance endogenous fixation of neurologically harmed tissues, and reduce apoptosis or cell defeat in hippocampus of epileptic rodents [71]. Aside from this, the anti-inflammatory response of MSCs impeded IL-1β over-expression in epilepsy that stifled GABA discharge to some degree through hindrance of NF-κB signaling pathway [72].

Our examination clarified that carbamazepine could significantly advance brain GABA content. Higuchi et al. [73] elucidated that CBZ can arouse GABA contents in kindled rodents temporal cortex. Moreover, Nagaki et al. [74] delineated that brain GABA level is significantly elevated in CBZ-treated rodents. Dated back, Battistin et al. [75] assessed the anti-convulsant drugs impact on GABA brain level and the activities of GAD67 or GABA aminotransferase (GABA-T) enzymes. These investigators discovered the elevation of

GABA level on utilizing CBZ treatment however no effect on enzymes activities.

One conceivable neurotransmitter candidate for CBZ therapy is GABA [76]. Bernasconi et al. [77] cited that CBZ decreases GABA turnover in animals brain as valproic acid through inhibition of succinic semialdehyde dehydrogenase, a mitochondrial enzyme that decays GABA into succinate [78]. Furthermore, CBZ and oxcarbazepine are distinguished to inspire GABA-A receptor (GABA-AR) and limit NMDA activated glutamate receptors or neuronal nicotinic acetylcholine receptors (nAChRs) [76]. Lastly, CBZ possesses anti-inflammatory impact *via* diminishing IL-1ß over-expression that restrains GABA discharge [79].

In the present investigation pilocarpine administration has been found to evok significant dwindle in brain Sema 4D level. Barnes et al. [80] recognized that semaphorins levels are droped in kainic acid (KA)- prompted epileptic rodents that might participate in axonal sprouting post SE. In addition, Xia et al. [81] mentioned that seizure induces changes in expression of axon guidance proteins (semaphorins) in rat models of epilepsy leading to the wiring of neuronal circuits all through later phases of epilepsy, adding to its progression to chronic nature.

Semaphorins are believed as a huge family of axon guidance cues [82]. Strikingly, semaphorin4D (Sema4D) is a class 4 transmembrane type of semaphorins that proteotically isolated from the neuronal surface and the membrane conjugated part at postsynaptic membranes. It is known to be fundamental for synapse development, supporting a way for Sema 4D signaling control that is taken an interest in the pathogenesis of illnesses recognized by shrewd imperfections of synaptic transmission, including epilepsy [83]. Luo et al. [84] illuminated that pilocarpine prompted-SE causes oligodendrocytes degeneration in the rodent hippocampus amid the acute stage, and the quality of this misfortune continued through epileptogenesis, diminishing Sema4D expression. It was perceived that knockdown of Sema4D in postsynaptic neuron resulted in adwindle of GABAergic synapses density framed onto this neuron, with no impact on density of glutamatergic synapses [85]. Sema4D is one of the little molecules that favorably control GABAergic synapse development by PlexinB1 that recruiting molecules to both presynaptic alongside postsynaptic terminals, ie. Sema 4D on the postsynaptic key neuron ties PlexinB1 on the presynaptic inhibitory neuron, creating a transsynaptic signals modifying GABAergic synapse formation [86]. In addition, Kuzirian et al. [87] depicted that treatment of an organotypic hippocampal slice an *in vitro* epileptic model with Sema4D seriously suppresses neuronal hyperexcitability by means of a shift in the excitation-inhibition balance where it viewed as a positive screen for functional GABAergic synapse development in hippocampal neuronal culture. Acker et al. [88] examined Sema4D capacity to invigorate new GABAergic synapse development and govern seizure action in adult mice. They observed the capability of Sema4D to hinder network hyperexcitability proposing its conceivable use as a novel therapy for epilepsy.

On the contrary, a significant expansion in brain Sema4D content has been enrolled in chronic epileptic groups transplanted with BMSCs or ADMSCs in this study. Assunção-Silva et al. [89] outlined that proteomic arrangement of the secretome for MSCs derived from fat tissue and bone-marrow as well as umbilical cord is accomplished, where proteins relevant for neuroprotection, neurogenic, neuro-differentiation, axon guidance and growth capacities are perceived. Besides, Fayyad-Kazan et al. [90] screened numerous populaces of human MSCs for their transcription profiles of a distinguished arrangement of immuno-modulatory genes. They cited that galanin and Sema4D are pioneer immuno-regulatory genes transcribed in an assortment of MSCs. Additionally, their records uncovered that inflammation-priming of either ADMSCs or BMSCs animates Sema4D in addition to Sema7A transcription.

Mesenchymal stromal cells implantation elicited neuroprotection and also recovery in the damaged regions of numerous trial rodent models [91]. Therapeudic impacts of MSCs may incorporate at least one of the consequent systems involving: I) MSCs differentiation capacity into proficient mature neurons or oligodendrocytes (MSC plasticity); II) MSCs consequences for endurance of hurt neurons or oligodendrocytes (neuroprotection) [92] that may raise Sema4D expression.

Treatment of epileptic control group by CBZ in the present setting rendered a significant enhancement in brain Sema4D concentration. It was discovered that CBZ blocks oligodendrocytes cytotoxicity through hampering of microglial creation of NO and ROS [93]. Prominently, glutamate interceded excitoxicity develops depolarization of oligodendrocytes with an ensuing ascent in Na^+ deluge which can drive turn around Na^+-Ca^{2+}-exchanger (NCX) expanding Ca^{2+} inundation, that actuate their apoptosis [94]. Sodium channel blockers (carbamazepine) can stop this attack of glutamate on the oligodendrocytes, thus exhibiting a defensive impact on CNS axons damages [95]. Moreover, Cunha et al. [96] commented a neuroprotective impact for the day by day administration of CBZ in a pilocarpine-induced SE supporting the data that formerly showed by Capella and Lemos [97]. Similarly, Park et al. [98] investigation have demonstrated the defensive impact of CBZ on seizure-incited neuronal damage.

The results of our investigation revealed that pilocarpine attributed significant minimization of brain galanin level. Galanin (GAL) is viewed as an inhibitory neuropeptide that assume a crucial function in sorting out the harmony among inhibition as well as excitation in the brain *via* controlling libration of excitatory glutamate neurotransmitter from neuronal pre-synaptic terminals [99]. Lu et al. [100] commented that status epilepticus exhaust galanin from axonal projections of various neurons of hippocampus. Pilocarpine-stimulated seizures creates maladaptive varieties in expression of assorted neuropeptides, it drains the inhibitory peptides involving galanin, somatostatin and neuropeptide Y, and also improved expression of the pro-convulsant for example, tachykinins, substance P in addition to neurokinin B [101]. Likewise, Lundström et al. [102] have demonstrated that galanin expression in the hippocampus region is altered by seizures as well as on the contrary, exogenous galanin administration weakens seizure severity in hippocampus. Taken together these verifications prescribed that galanin may in fact consider as an anti-convulsant peptide in addition to hippocampal galaninergic innervation fatigue might take an interest in the seizures evolution.

Additionally, galanin is co-expressed with principal neurotransmitters in various brain systems adjusting synaptic neurotransmission [103]. Keimpema et al. [104] instituted that galanin is freed from GABAergic nerve endings to cholinergic neurites controlling cholinergic neuritogenesis and additionally affecting cytoskeletal integrity as well as dynamics. On the other side, SE animates deterioration of GABAergic interneurons and inhibitory synapses in hippocampus [105] which thus will suppress galanin expression.

Our outcomes enlightened that implantation of BMSCs or ADMSCs yielded significant elevation in brain galanin content. Mesenchymal stromal cells are equipped for generating neurotrophic factors participating in safeguarding and in addition renovating of harmed nervous tissue [106]. It was showed that MSCs can reduce nerve damage prompted changes in galanin and neurpeptide Y expression [107]. Furthermore, Jones et al. [108] examined the conveyance of galanin peptide in the early mouse embryo and outlined that galanin peptide profoundly abundant in mesenchymal as well as neural crest tissues. In addition, Louridas et al. [109] illustrated that galanin mRNA and protein are significantly expressed in BMSCs cultures up to 4 passages. Moreover, BMSCs express galanin receptors, and the galanin receptor 2 (GalR2) denoted the pre-eminent flow. On the other side, it has been found that raised serum galanin content in a galanin transgenic rodent enhances the transportation of infused BMSCs *in vivo* to harmed tissue. These clarifications propose the function of galanin as an essential growth factor contributed not only in the BMSCs homing to harmed tissue most likely through GalR2 actuation, but also in the fixation of harmed brain. As well, Fayyad-Kazan et al. [90] set up that galanin is expressed in numerous kinds of MSCs, it was exceedingly expressed in foreskin MSCs and in ADMSCs.

The current research perceptions showed that carbamazepine drug would augment brain galanin concentration significantly. Numerous confirmations demonstrated that CBZ has neuroprotective features that guard GABAergic interneurons expressing galanin neuropeptide [105]. CBZ played out an incredible neuroprotective activity against neuronal destruction in parkinson's disorder initiated by rotenone *via* up-regulation of BDNF [110]. Also, CBZ in a

dose dependant manner frustrated the seizure-initiated hippocampal sclerosis and diminish neurological shortages in kainic acid (KA) challenged rodents [111]. In addition, CBZ elevated stat3 phosphorylation hoisting Bcl-xL and Bcl-2 transcription in the brain that have been suggested as key effectors of neuronal endurance in the hippocampal CA3 area of kainic acid challenged mice [98].

Injection of *Wistar* rodents in the current attempt with pilocarpine induced significant down-regulation of hippocampal synapsin-I mRNA level. Synapsin-I (Syn-I) is a synaptic vesicle associated protein identified with control of synaptogenesis, neurotransmitter discharge as well as foundation of a synaptic vesicle reserve pool [112]. Wu et al. [113] noticed that synapsin-I is decreased obviously in the epilepsy group that was invigorated partly after valproate administration. Moreover, Damaye et al. [114] immuno-histochemistical records indicated that the expression of synapsin-I is blunted significantly in the hippocampus of epileptic groups. Further, Zhang et al. [115] showed that debilitate of synapsin-I gene expression significantly bring the epileptic discharge up in mice through the reduction of inhibitory synaptic transmission.

Ever since neurotransmitter discharge has a basic control role, it is theorized that synapsin-I expression may be down-regulated through feedback regulation. Synapsin-I expression can be decreased as a result of reduced inhibitory synaptic transmission in light of the fact that synapsin-I is progressively copious at inhibitory GABAergic synapses [114]. Besides, Cesca et al. [116] demonstrated that the cancellation of Syns in mice extremely hinders inhibitory transmission.

Additionally, the production [117] and phosphorylation [118] of synapsin-I are motivated by means of BDNF that is a neuroprotective factor related with neuronal signaling, endurance and plasticity [119]. On the opposite side, pilocarpine has the ability to promote free radicals production, that in turn inhibit the functions and/or levels of BDNF and its synaptic plasticity effectors as synapsin-I and cAMP response element binding protein (CREB) [120]. Furthermore, a significant decline in the levels of the synapse associated proteins involving synapsin-I after endoplasmic reticulum (ER) stress has been detected [121].

Pilocarpine generates drawn out and un-manageable SE which is related with neurodegenerative processes including synaptic malfunction and additionally gliosis, the most conceivable systems for advanced neuronal harm [122]. In parallel to this, the synaptic structure was destroyed, and the quantity of synapses was diminished [123] resulting in marked shrinkage in synapsin-I level [124].

The infusion of the two studied types of MSCs in epileptic group have been established to upgrade mRNA level of synapsin-I gene as shown in our results. Yang et al. [125] showed that human MSCs administration to Alzheimer rodent model restor cognitive capacity, lifted synapsin-I level and significantly diminished Aß deposition. Similarly, Bae et al. [126] illustrated that dynamin-1 and synapsin-I expression, the cheif pre-synaptic proteins identified with synaptic transmission, are significantly improved in AD mice infused with BMSCs. From other point of view, Cao et al. [127] manifested that MSCs implantation are fit for expressing synapsin-I at various developmental stages. Chen et al. [123] explained that subsequent BMSCs injection, the cell number expressing synapsins in the injured spinal cord was improved and additionally the fluorescence intensity is enhanced which demonstrated that BMSCs can expand expression of synapsins.

Zhang et al. [128] mentioned that hoisted expression level of synapsin-I after MSCs infusion can be in consequence of neurotrophic factors discharge, *in vivo* incitement of neural stem cells differentiation and improved number of CNS neurons and synapses. Furthermore, Oh et al. [129] outlined that intravenous injection of MSCs in mice empowered neurogenesis *via* stimulation of Wnt/ß-catenin signaling. Moreover, MSCs infusion has been shown to significantly enhance the activity of superoxide dismutase (SOD), a key enzyme responsible for tumbling oxidative stress in the cell [130] and suppress malondialdehyde (MDA) level, whichever is belonged to oxidative tissue injury [131]. Besides, *in vitro* and *in vivo* studies uncovered that MSCs produce nerve growth factor (NGF), vascular endothelial growth factor in addition to BDNF which can induce neurogenesis in the hippocampus [132] that provoke synapsin-I expression.

Implantation of BMSCs has been found to increase the expression of choline acetyl transferase, glutamic acid decarboxylase and synapsins following CNS damage, illustrating that BMSCs can elevate neuronal number, encourage revival of neural capacities as well as progress the production of acetylcholine, GABA or other neurotransmitters that improve the synaptic structure [133].

The current data designated that synapsin-I mRNA level is upgraded in the epileptic group treated with CBZ. A typical mechanism for CBZ activity in the treatment of bipolar disease could incorporate neuroprotection interceded by up-regulation of brain BDNF in addition to Bcl-2 expression [134]. Transcription of BDNF is controlled fundamentally by means of cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) [135] that must be phosphorylated to pCREB in order to transcribe CREB-regulated genes, including BDNF [136]. A prior *in vitro* study affirmed that CBZ encourages CREB inspiration in human neuroblastoma SH-SY5Y cells that increment pCREB in rodent brain [137] in this manner it increases BDNF as well as Bcl-2 mRNA levels in rodent frontal cortex [138]. Raised BDNF level may assist to avoid the revealed abatements in neuronal and glial numbers related with neurological ailments, that attributed to the decrease of BDNF and other neurotrophic factors levels [139]. Moreover, arachidonic acid signaling interferes with the transcription of neuronal survival factors, while decrease of this signal *via* chronic administration of CBZ oppositely affect BDNF [140] that will build up expression of synapsin-I gene.

Hippocampus is a territory of interest to investigate the primary histopathological changes in pilocarpine-convinced seizures, as it is a standout amongst the most vulnerable brain areas for epilepsy-related brain injury and assumes a key role in the advancement and safeguarding of limbic seizures [141].

Steady with our investigation Wozny et al. [142] and Da Costa et al. [143] showed that pilocarpine-infused rodents exhibit trademark neurons degeneration in hippocampal CA1 pyramidal cell layer as well as cerebral cortex. In addition, Kuruba et al. [144] uncovered that 80% of neurons powerfully diminished in the hilus and in dentate gyrus as a consequent to pilocarpine-animated SE in mice. Furthermore, Cunha et al. [96] splashed that qualitative investigation of brain parts in pilocarpine-induced SE rats demonstrat extreme harm (cell misfortune) all through the hippocampus when contrasted with cell densities of control counterparts.

On the contrary, Salem et al. [55] cleared up that BMSCs implantation disclosed intact architecture of neurons in the hippocampus of BMSCs-transplanted rodents subsequent to examination of the hippocampal CA1 area after pilocarpine injection. Chung et al. [145] sprinkled that adipose mesenchymal stromal cells downsize neuronal defeat that might be related to the block of BBB unsettling influence, reduce endothelial harm and decline in neutrophil invasion. The favorable impacts of MSCs on brain illness have been elucidated as a result of various cytokines or chemokines initiated through MSCs treatment, rather than *via* the perseverance and differentiation of grafted cells [146]. The mechanism underlying this activity is recommended for the neuroprotective impact of neurotrophic factors discharged by them, for example, BDNF, SDF-1, insulin-like growth factor, VEGF, and glial cell line-derived neurotrophic factor, other than raised endogenous neurogenesis, in addition to the change of the inflammatory or immune reactions [147].

Additionally, Cunha et al. [96] depicted a fractional neuroprotective impact of the everyday supplemented CBZ, in like manner that formerly appeared by Capella and Lemos, [97]. Furthermore, previous investigators have recommended the neuroprotective impacts of CBZ on ischemic/hypoxic model of neuronal harm [148].

Conclusion

The present investigation clarified that MSCs have made favourable achievement in the recovery of spontaneous recurrent seizures characterized chronic epilepsy. Stem cell transplantation may represent the first realistic strategy for controlling seizures burden on brain areas. Neuroprotective activity of MSCs was accomplished *via* manipulated epileptic neuronal circuit, releasing neurotrophic factors and regains the synaptic plasticity which are considered as the potential mechanisms of MSCs in the treatment of chronic epilepsy. This could provide clues for the therapeutic effect of MSCs on focal epilepsies from multiple prespections. This study opens up aline of

thought about the development of MSCs therapy in epileptic patients.

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