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New approaches in the horizon for treatment of Alzhimer's disease

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ABSTRACT

The focus of our interest was to explore the role of single intravenous dose of mesenchymal stem cells (MSCs) derived from bone marrow (BM-MSCs) and adipose tissue (ADMSCs) in management of Alzheimer's disease (AD) in comparison with cerebrolysin as a reference drug in experimental model. The animals included in the study were divided into five groups; the first one served as healthy control, while, the other groups were administered with $AlCl_3$ orally to induce AD. Then, group of AD rats were left without treatment (Group 2) and the other groups were treated with cerebrolysin (Group 3), BM-MSCs (Group 4) and ADMSCs (Group 5). The levels of TGF-β1, MCP-1 and BDNF were determined in serum by ELISA. Nestin gene expression level was detected in brain tissues by sqRT-PCR. While, ChAT expression was determined in brain tissue by immunohistochemical procedure. Also, histopathological examination of brain tissues was performed. BM-MSCs and ADMSCs were engrafted into AD affected brains and caused insignificant decline in serum TGF-β1 and MCP-1 levels in concomitant with significant elevation in serum BDNF and brain nestin gene expression levels. Furthermore, the engrafted cells elecited significant increase in the expression of brain ChAT and ameliorated the neurodegeneration of hippocampus. In conclusion, the current data provide distinct evidence about the importance of BM-MSCs and ADMSCs as a novel therapeutic avenue for AD through their anti-inflammatory effect, neurotrophic and neurogenic potentials as well as Aβ clearing activity.

Key words: Alzheimer's disease, mesenchymal stem cells, anti-inflammatory effect, neurotrophic capacity, neurogenic potential.

INTRODUCTION

Alzheimer's disease (AD) is the most well-known neurodegenerative disease featuring progressive impairments in memory, cognition, and behavior and ultimately leads to death [1]. It has numerous etiological factors including genetics, environmental factors, and general lifestyles [2]. The pathophysiological hallmarks of AD include extracellular β-amyloid protein (Aβ) deposition in the forms of senile plaques and intracellular deposits of the microtubule associated protein tau as neurofibrillary tangles (NFTs) [3]. A consequent cascade of events occurs as a result of Aβ aggregation and deposition, including an inflammatory response, free radical formation and oxidative stress. These processes contribute to neurotransmitter and synaptic dysfunction, suppression of neurotrophic factors, excitotoxicity and eventually, neuronal death [4].

Since, elevated Aβ deposition is the key pathogenic factor for AD and the main cause for neuronal loss in AD [5] and the current drug therapies for AD treatment are hindered due to poor efficacy and side effects [6], the promising therapeutic strategies for AD has focused on preventing, reversing, and reducing Aβ deposition [7]. Cerebrolysin is a neuropeptide preparation which mimics the action of endogenous neurotrophic factors on brain protection and repair. Furthermore, it has the ability to decrease the deposition of Aβ and the phosphorylation of microtubule-

associated protein tau through regulating glycogen synthase kinase-3β and cyclin-dependent kinase 5 activity, increase synaptic density and restores neuronal cytoarchitecture. These effects protect integrity of the neuronal circuits and thus result in improved cognitive and behavioral performance [8]. On the other hand, it has been reported that cell therapy is a potential therapeutic approach for neurodegenerative disorders [9].

Recently, many groups have demonstrated that stem cells have regenerative as well as paracrine effects [10-12]. Moreover, the studies of Kim et al. [13]; Bae et al. [14] and Ma et al. [15] revealed that transplanting mesenchymal stem cells derived from human umbilical cord, bone marrow and adipose tissue into the brains of Alzheimer's transgenic animals decrease Aβ deposition, amyloid precursor protein (APP) generation, and microglia activation leading to improvement in cognitive and memory performances and neuronal survival.

It has been reported that local stem cell delivery causes bleeding and tissue injury [16, 17]. Based on, the current study sought to clarify the mechanisms involved in repressing the neurodeterioration cascade in chemically induced AD by single intravenous dose of MSCs in comparison with multiple doses of cerebrolysin drug.

MATERIALS AND METHODS

Mesenchymal stem cells

Mesenchymal stem cells were isolated from bone marrow and adipose tissue of 6-week-old male *Sprague Dawley* rats. Thereafter, they were characterized morphologically by inverted microscope examination, PCR detection of CD14, CD29, CD34, CD44, CD45 and CD106 gene expressions and *in vitro* differentiation into adipocytes, chondrocytes and osteocytes according to previously published work [18, 19].

Chemicals and drugs

- Aluminum chloride was provided from BDH Laboratory Supplies, England.
- Cerebrolysin® ampoules were purchased from EBEWE Pharma, Austria.

Experimental design

Forty adult female *Sprague-Dawley* rats weighing 130-150 g were obtained from the Animal House Colony of the National Research Centre, Giza, Egypt and acclimatized in a specific area where temperature 25±1ºC and humidity 55%. Rats were controlled constantly with 12 h light/dark cycles at National Research Centre, Animal Facility Breeding Colony. Rats were individually housed with *ad libitum* access to standard laboratory diet consisted of casein 10%, salt mixture 4%, vitamin mixture 1%, corn oil 10%, cellulose 5% and completed to 100 g with corn starch [20] and tap water. Also, they were cared for according to the guidelines for animal experiments which were approved by the Ethical Committee of Medical Research at National Research Centre, Giza, Egypt.

The animals were classified into 5 groups (8 rats/group). The first group was healthy control group. The groups from second to fifth were orally administered with aluminum chloride ($AlCl_3$) in a dose of 17 mg/kg b. wt. [21], daily for 75 days for induction of AD disease. Then, the second group was left without treatment for 2 months; the third group was treated intraperitoneally with cerebrolysin in a dose of 1.08 ml/kg b. wt. that is equivalent to the recommended human dose [22] according to Barnes and Paget [23] equation, 5 days/week for one month and thereafter two times per week for another month. The fourth and fifth groups were infused intravenously with a single dose of 3 x 10^6 cells/rat of BM-MSCs and ADMSCs respectively [24]. In brief, the AD induced rats were deeply anaesthetized *via* diethyl ether and MSCs were suspended in 100 µL PBS before transplantation and then slowly injected into the tail vein in 5 min with a 27G needle. The needle was kept in the tail vein for another 5 min to avoid regurgitation and then withdrawn.

At the end of the experimental period, all animals were fasted for 12 h and the blood samples were collected from retro-orbital venous plexus under diethyl ether anaesthesia. The blood samples were left to clot and the sera were separated using cooling centrifugation (4ºC) at 1800 x g for 10 min and then stored immediately at -20ºC in clean plastic Eppendorf until analyzed. Meanwhile, the whole brain of each rat was rapidly and carefully dissected. Then, each brain was sagitally divided into two portions. The first portion was immediately frozen in liquid nitrogen and stored at −80º C prior to extraction for molecular study. While, the second portion was fixed in formalin buffer (10%) for immunohistochemical examination and histological investigation.

Detection of male-derived MSCs in the brain of females

The genomic DNA was isolated from the brain tissue of female rats which were treated with BM-MSCs and ADMSCs using phenol/chloroform extraction and ethanol precipitation method according to Sambrook et al. [25] with minor modifications. The presence or absence of the sex determination region on the Y chromosome (SRY) gene in recipient female rats was assessed by PCR technique. Primer sequences for SRY gene (accession no.

NC_000087.7; forward 5′-CATCGAAGGGTTAAAGTGCCA-3′, reverse 5′-ATAGTGTGTAGGTTGTTGTCC-3′) were obtained from published sequences [26] and amplified to a product of 104 bp. The PCR conditions were as follows: incubation at 94 °C for 4 min; 35 cycles of incubation at 94 °C for 50 s, 60 °C for 30 s, and 72 °C for 1 min; with a final incubation at 72 °C for 10 min. PCR products were separated using 2% agarose gel electrophoresis and stained with ethidium bromide.

Biochemical assays

Serum transforming growth factor β1 (TGF-β1) level was assayed by enzyme linked immunosorbent assay (ELISA) technique using kit purchased from DRG Diagnostics Co., Germany, according to the method described by Kropf et al. [27]. While, serum monocyte chemoattractant protein 1 (MCP-1) level was determined by ELISA method using kit purchased from Bender MedSystems GmbH, Europe, according to the method described by Baggiolini et al. [28]. Serum brain derived neurotrophic factor (BDNF) level was determined by ELISA technique using kit purchased from Millipore corporation, USA, according to the method described by Laske et al. [29].

Semi-quantitative real time PCR (sqRT-PCR) detection of nestin gene expression

Total RNA was isolated from brain tissue of female rats by the standard TRIzol® reagent extraction method (Invitrogen, USA). Then, the complete Poly(A)$^+$ RNA was reverse transcribed into cDNA in a total volume of 20 µL using RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). An amount of total RNA (5µg) was used with a reaction mixture, termed as master mix (MM). The MM consisted of 50 mM $MgCl_2$, 5x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3; 10 mM of each dNTP, 50 µM oligo-dT primer, 20 U ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity) and 50 U M- MuLV reverse transcriptase. The RT reaction was carried out at 25 °C for 10 min, followed by 1 h at 42 °C, and the reaction was stopped by heating for 5 min at 99 °C. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through sqRT-PCR. An iQ5-BIO-RAD Cycler (Cepheid, USA) was used to determine the rat cDNA copy number. PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd., Germany), 0.5 µL 0.2 µM forward primer, 0.5 µL 0.2 µM reverse primer, 6.5 µL distilled water, and 5 µL of cDNA template. Primer sequences were as follows: nestin: (accession no. NM_012987.1) F: 5′-TGGAGCGGGAGTTAGAGGCT-3′, R: 5′-ACCTCTAAGCGACACTCCCGA-3′ [30] and β-actin: (accession no. NM_031144.3) F: 5′-CTGTCTGGCGGCACCACCAT-3′, R: 5′-GCAACTAAGTCATAGTCCGC-3′ [31]. The reaction program was allocated to 3 steps. First step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle divided to 3 steps: (a) denaturation at 95.0°C for 15 sec; (b) annealing at 55.0°C for 5 sec and 60°C for 30 sec for nestin and β-actin genes respectively and (c) extension at 72.0°C for 30 sec.

Immunohistochemical (IHC) examination of brain choline acetyltransferase (ChAT) expression

Formalin fixed brains were washed in tap water and ascending grade of ethyl alcohol (30, 50, 70, 90% and absolute) was used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 ^oC in hot air oven for 24 h. Sections were cut into 4µ thick by slidge microtome then fixed on positive slides in a 65 ^oC oven for 1 h. Slides were placed in a coplin jar filled with 200 mL of triology working solution (Cell Marque, CA-USA) which combines the three pretreatment steps: deparaffinization, rehydration and antigen unmasking. Then, the jar is securely positioned in the autoclave with the temperature adjusted to reach 120 ^oC and maintained stable for 15 min after release of pressure. After cooling for 30 min, sections were washed and immersed in Tris-buffer saline (TBS) to adjust the pH. Then, quenching endogenous peroxidase activity was performed by immersing slides in 3% hydrogen peroxide for 10 min. Broad spectrum LAB-SA detection system (Invitrogen, USA) was used to visualize any antigen-antibody reaction in the tissue. Background staining was blocked by putting 2-3 drops of 10% goat non immune serum blocker on each slide and incubating them in a humidity chamber for 10 min. Excess serum was drained and two-three drops of the working solution (1:100) of the primary antibody for ChAT (LifeSpan BioSciences Inc., USA**)** were applied and the slides were incubated in the humidity chamber overnight at 4 ^oC. Henceforward, biotinylated secondary antibody was applied on each slide for 20 min followed by 20 min incubation with the streptavidin horse reddish peroxidase (HRP) enzyme conjugate and then 2-3 drops of 3,3′-diaminobenzidine (DAB) chromogen were applied on each slide for 2 min. DAB was rinsed, after which counterstaining with Mayer hematoxylin and cover slipping were performed as the final steps before slides were examined under the light microscope (Olympus Cx21 with attached digital camera) [32]. Image analysis was performed using the image J, 1.41a NIH, (USA) analyzer.

Histopathological investigation of brain tissue of rats

After fixation of brain tissues in 10% formalin buffer for 24 h, the tissues were washed in tap water and dehydrated by using ascending grade of ethyl alcohol (30, 50, 70, 90% and absolute). Specimens were cleared in xylene and embedded in paraffin at 56 ^oC in hot air oven for 24 h. Then, paraffin wax tissue blocks were prepared for sectioning

at 4 µ thick, collected on glass slides, deparffinized and stained by hematoxylin and eosin stain [33] for histopathological examination through the electric light microscope.

Statistical analysis

In the present study, all results were expressed as Mean ± S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 14 followed by least significant difference (LSD) to compare significance between groups [34]. Difference was considered significant when P value was <0.05.

RESULTS

BM-MSCs and ADMSCs homing

The agarose gel electrophoresis from DNA fragments **(Fig. 1)** showed that the sex determination region on the Y chromosome; SRY gene is found in the brain tissues of AD groups injected with BM-MSCs and ADMSCs. Meanwhile, this gene is not found in the brain tissues of healthy control rats.

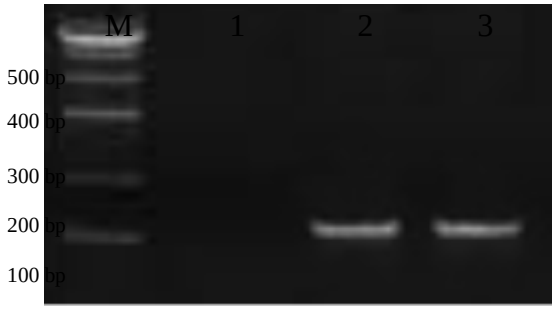

Fig. 1: An agarose gel electrophoresis showed SRY gene in AD rats treated with BM-MSCs and ADMSCs. M: represented DNA ladder. Lane 1: represented healthy control sample. Lane 2: represented sample from AD group treated with BM-MSCs. Lane 3: represented sample from AD group treated with ADMSCs

Impact of cerebrolysin, BM-MSCs and ADMSCs on serum TGF-β1, MCP-1 and BDNF levels

Table (1) showed that $AlCl_3$ administration elevated serum TGF-β1 (by 30.44%) and MCP-1 (by 22.1%) and depleted serum BDNF (by 18.23%) levels significantly (P< 0.05) relative to the healthy control group. Otherwise, treatment of $AlCl_3$ intoxicated rats with cerebrolysin, BM-MSCs or ADMSCs diminished serum TGF-β1 (by 7.31%, 6.14% and 5.25% respectively) and MCP-1 (by 6.39%, 6.28% and 3.97% respectively) levels insignificantly (P>0.05) when compared with the group of rats left untreated. At the same time, all treatments elevated serum BDNF level significantly (P<0.05) by 14.58% for cerebrolysin, 14.27% for BM-MSCs and 12.5% for ADMSCs in comparison with the group of rats left untreated.

Table (1): Serum TGF-β1, MCP-1 and BDNF levels in AD model rats treated with cerebrolysin, BM-MSCs or ADMSCs

Groups	TGF-β1 (pg/mL)	MCP-1 (pg/mL)	BDNF (pg/mL)
Healthy control	372.2 ± 2.9	74.3 ± 1.1	3943 ± 56.4
AD untreated	485.5 ± 10.9[a]	90.7 ± 1.1[a]	3224 ± 118.0[a]
AD + cerebrolysin	450.0 ± 17.0	84.9 ± 2.7	3694 ± 90.9[b]
AD + BM-MSCs	455.7 ± 11.7	85.0 ± 2.2	3684 ± 45.2[b]
AD + ADMSCs	460.0 ± 5.0	87.1 ± 1.9	3627 ± 51.7[b]

[a]Significant change at P< 0.05 in comparison with the healthy control group.
[b]Significant change at P< 0.05 in comparison with the AD untreated group.

Impact of cerebrolysin, BM-MSCs and ADMSCs on brain nestin gene expression level

Table (2) clarified that $AlCl_3$ administration down-regulated the expression level of nestin gene in brain significantly (P<0.05) by 48.46% *versus* the healthy control group. While, treatment of $AlCl_3$ intoxicated rats with cerebrolysin, BM-MSCs or ADMSCs up-regulated the expression level of nestin gene in brain significantly (P<0.05) by 62.69%, 79.10% and 71.64% respectively as compared with the group of rats left untreated. Furthermore, injection of $AlCl_3$ intoxicated rats with BM-MSCs or ADMSCs up-regulated the expression level of nestin gene in brain significantly (P<0.05) relative to those injected with cerebrolysin. Also, injection of $AlCl_3$ intoxicated rats with ADMSCs down-regulated brain nestin gene expression level as compared with those injected with BM-MSCs.

Table (2): Brain nestin gene expression level in AD model rats treated with cerebrolysin, BM-MSCs or ADMSCs

Groups	Relative expression of nestin gene (Nestin/β-actin)
Healthy control	1.30 ± 0.02
AD untreated	0.67 ± 0.004[a]
AD + cerebrolysin	1.09 ± 0.004[b]
AD + BM-MSCs	1.20 ± 0.005[bc]
AD + ADMSCs	1.15 ± 0.01[bcd]

[a]Significant change at P< 0.05 in comparison with the healthy control group.
[b]Significant change at P< 0.05 in comparison with the AD untreated group.
[c]Significant change at P< 0.05 in comparison with the AD + cerebrolysin group.
[d]Significant change at P< 0.05 in comparison with the AD + BM-MSCs group.

Impact of cerebrolysin, BM-MSCs and ADMSCs on brain ChAT expression

The data in **Table (3)** and optical micrographs in **Fig. (2)** demonstrated that $AlCl_3$ administration decreased the number of ChAT expressing cells in brain tissue significantly (P<0.05) by 50.83% as compared with the healthy control group. While, treatment of $AlCl_3$ intoxicated rats with cerebrolysin, BM-MSCs or ADMSCs increased the number of ChAT expressing cells in brain tissue significantly (P<0.05) by 36.18%, 48.11% and 45.86% respectively in comparison with the group of rats left untreated.

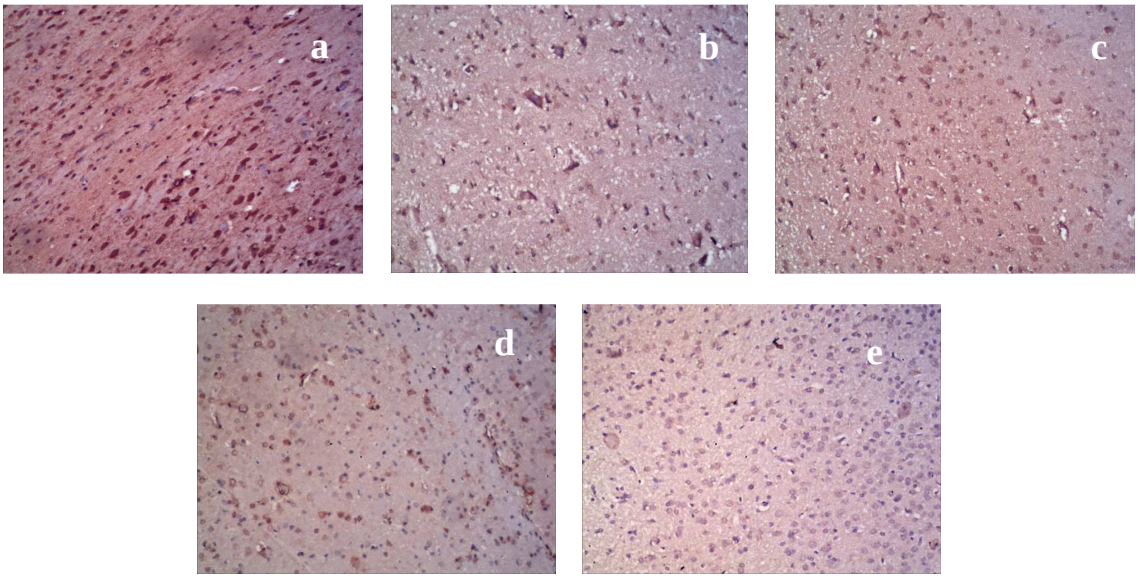

Fig. 2: Immunohistochemical examination of ChAT expression in AD model groups. (a): Healthy control, (b): AD untreated, (c): AD + cerebrolysin, (d): AD + BM-MSCs and (e): AD + ADMSCs

Table (3): Number of ChAT expressing cells in brain of AD model rats treated with cerebrolysin, BM-MSCs or ADMSCs

Groups	Number of ChAT expressing cells		
Healthy control	290.0	±	2.7
AD untreated	142.6	±	6.1[a]
AD + cerebrolysin	194.2	±	10.3[b]
AD + BM-MSCs	211.2	±	7.2[b]
AD + ADMSCs	208.0	±	5.4[b]

[a]Significant change at P< 0.05 in comparison with the healthy control group.
[b]Significant change at P< 0.05 in comparison with the AD untreated group.

Histopathological alterations in brain tissues of female rats

The optical micrograph of a cross-sectioned brain tissue of healthy control group showed normal histological structure of the hippocampus as recorded in **Fig. (3a)**. While, the optical micrograph of a cross-sectioned brain tissue of $AlCl_3$ administered group showed encephalomelacia with plaques formation in the hippocampus **(Figs. 3b and 3c)**. On the other hand, the optical micrographs of cross-sectioned brain tissues of $AlCl_3$ intoxicated rats treated with cerebrolysin, BM-MSCs or ADMSCs showed normal histological structure of the hippocampus **(Figs. 3d, 3e and 3f** respectively**)**.

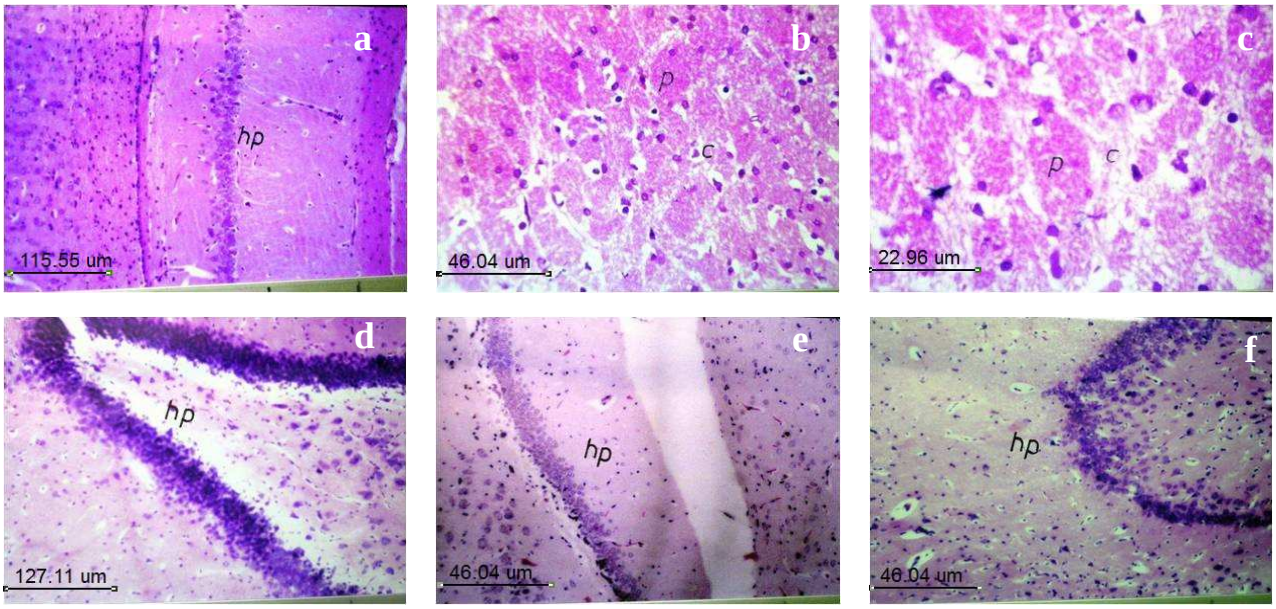

Fig. 3: Optical micrograph of a cross-sectioned brain tissue of (a): healthy control group shows normal histological structure of the hippocampus (hp); (b and c): untreated AD group shows encephalomelacia (c) with plaques formation (p) in the hippocampus and (d – f): AD group treated with cerebrolysin, BM-MSCs or ADMSCs shows normal histological structure of the hippocampus (hp) respectively

DISCUSSION

The capability of adult MSCs to transdifferentiate into neural cell types has aroused great interest in research. Such capacity opens extensive possibilities for using MSCs as a therapeutic approaches in a variety of neurological disorders [35]. In the present study, we investigate the potency of a single dose of intravenously transplanted MSCs to restrain the neurodegeneration in experimental model of AD after 2 months. For this purpose, AD was induced chemically in experimental animals by oral administration of $AlCl_3$ for 75 days.

Various lines of evidences have indicated that intravenously transplanted MSCs could migrate and integrate like neural stem cells in the brain [36, 37]. In consistent with these studies, the data of the current work demonstrated that the intravenously transplanted MSCs either derived from BM-MSCs or ADMSCs had the ability to migrate towards injured brain tissues. This feature could be allied to MSCs broader expression of homing molecules [38]. More in details, Ji et al. [39] as well as Karp and Teo [40] have reported that MSCs express chemokine C-C motif receptor 2 (CCR2) that interact with MCP-1 and promote their engraftment. Moreover, another study has demonstrated that upon transmigration, MSCs secrete matrix metalloproteinases to degrade the endothelial basement membrane and facilitate MSCs journeying toward chemotactic agents [41].

The current data indicated that $AlCl_3$ administration increased serum TGF-β1 and MCP-1 levels significantly. These findings are in conformity with the previous studies of Chao et al. [42] and Galimberti et al. [43]. Accumulated evidences have suggested that Aβ deposition play a crucial role in AD development as it originates a chronic inflammatory response, which likely contributes to neuronal death [44]. Furthermore, Pratico et al. [45] reported that intoxication with Al could induce the formation of reactive oxygen species (ROS) which in turn increase brain oxidative stress and lipid peroxidation that promote the formation and deposition of Aβ peptide. These Aβ peptides aggregate to form fibrillar deposits that triggers inflammatory reactions and activates microglia in AD brain. It has been reported that activated microglia cells which are localized to fibrillar plaques produce TGF-β1 mRNA and protein in response to activation by the usual cohort of pro-inflammatory signals [46]. Over and above, Porcellini et al. [47] reported that activated microglia express a large number of beta chemokines including MCP-1. The increase in the efflux of cytokines from brain to peripheral blood supply [48] could explain the elevation in the level of serum TGF-β1 and MCP-1.

In the light of the current data, treatment of AD rats with cerebrolysin for 2 months reduced the serum level of TGF-β1 and MCP-1. This effect might be ascribed to the capability of cerebrolysin to reduce Aβ deposits as well as astroglial activation as reported by Xing et al. [49]. Furthermore, our data showed after 2 months from treatment of AD rats with BM-MSCs or ADMSCs decline in serum TGF-β1 and MCP-1 levels. Growing body of evidence has suggested that implanted MSCs could modulate the microglia/macrophage activation including inflammatory responses [50]. Additionally, Lee et al. [51] demonstrated that BM-MSCs have the ability to promote the reduction

of Aβ deposits and consequently suppress microglia activation. In the same line, it has been documented that adipose tissue derived MSCs have the ability to decrease Aβ level through their secretion of neprilysin [52]. Therefore, the observed potency of the transplanted MSCs to decline the estimated serum inflammatory markers in the current study could be allied to their ability to reduce Aβ deposition.

Earlier studies showed that BDNF expression is extremely diminished in the hippocampus and some cortical areas in AD patients [53]. Also, Angelucci et al. [54] stated that decreased serum BDNF level may reflect similar alterations in central nervous system. In line with these findings, the current data revealed that $AlCl_3$ administration decreased serum BDNF level significantly. This observation could possibly explained by the fact that accumulation of Al in the brain causes inhibition of BDNF functions as well as BDNF depletion, and that the exhaustion of BDNF promotes the neurotoxicity of Aβ protein and accelerates AD pathogenesis [55].

Treatment of AD rats with cerebrolysin produced significant increase in serum BDNF level. This finding comes in line with the previously reported data of Selianina and Karakulova [56] and it could possibly ascribed to that cerebrolysin is a mixture of several active peptide fragments and neurotrophic factors including BDNF as documented by Georgy et al. [57]. Also, in the light of the current data, injection with BM-MSCs or ADMSCs increased the level of serum BDNF significantly. This enhancing impact might be attributed to their ability to secrete and alter the expression of neurotrophic factors including the BDNF as reported by Jiang et al. [58] and Han et al. [59].

Accumulated evidences have suggested that cholinergic hypoactivity has a detrimental influence on neurogenesis [60]. Furthermore, it has been demonstrated that acetylcholine plays an important role in the brain as a growth-regulatory signal to promote the proliferation of neural stem cells [61]. In line with these studies, our results revealed that $AlCl_3$ administration experienced significant decrease in the expression of brain ChAT and nestin gene. This effect could be related to the degeneration of cholinergic terminals in cortex and hippocampus associated with aluminum exposure as reported by Platt et al. [62].

In the current study, injection with cerebrolysin, BM-MSCs and ADMSCs increased the expression of brain ChAT and nestin gene significantly. The enhancing effect of cerebrolysin on these markers level might be imputed to its neurotrophic and neurogenic effects as attested by Rockenstein et al. [63]. While, the potency of MSCs to increase the expression of these markers could be assigned to their migration throughout the brain and differentiation into neurons and glial cells that express ChAT and nestin as reported by Balasubramanian et al. [64], Mezey et al. [65] and Park et al. [66].

The observed alterations in the estimated markers by $AlCl_3$ administration in the current work were confirmed pathologically by the presence of plaques formation in the hippocampus. This finding is greatly supported by that of Rodella et al. [67] who demonstrated that oral Al exposure causes the accumulation of beta amyloid protein and neurodegenerative damage in AD-model. On the other hand, there is no evidence of amyloid plaques deposition in the brain of the rats treated with cerebrolysin, BM-MSCs and ADMSCs. The Aβ clearing effect exerted by cerebrolysin administration could be attributed to its ability to regulate Aβ degradation and modulate APP expression, maturation, or processing as reported by Rockenstein et al. [68]. While, the observed clearance of Aβ due to treatment with MSCs could be explained by their ability to remove Aβ deposits and elevate the levels of Aβ-degrading enzymes as documented by Lee et al. [51] and Ma et al. [15].

CONCLUSION

The results of the current study lend further credence to the notion that MSCs are multipotent therapeutic approach that not only reduce inflammation and clear Aβ deposition, but also hold the potential to increase neurotrophic factors and enhance neurogenesis.

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