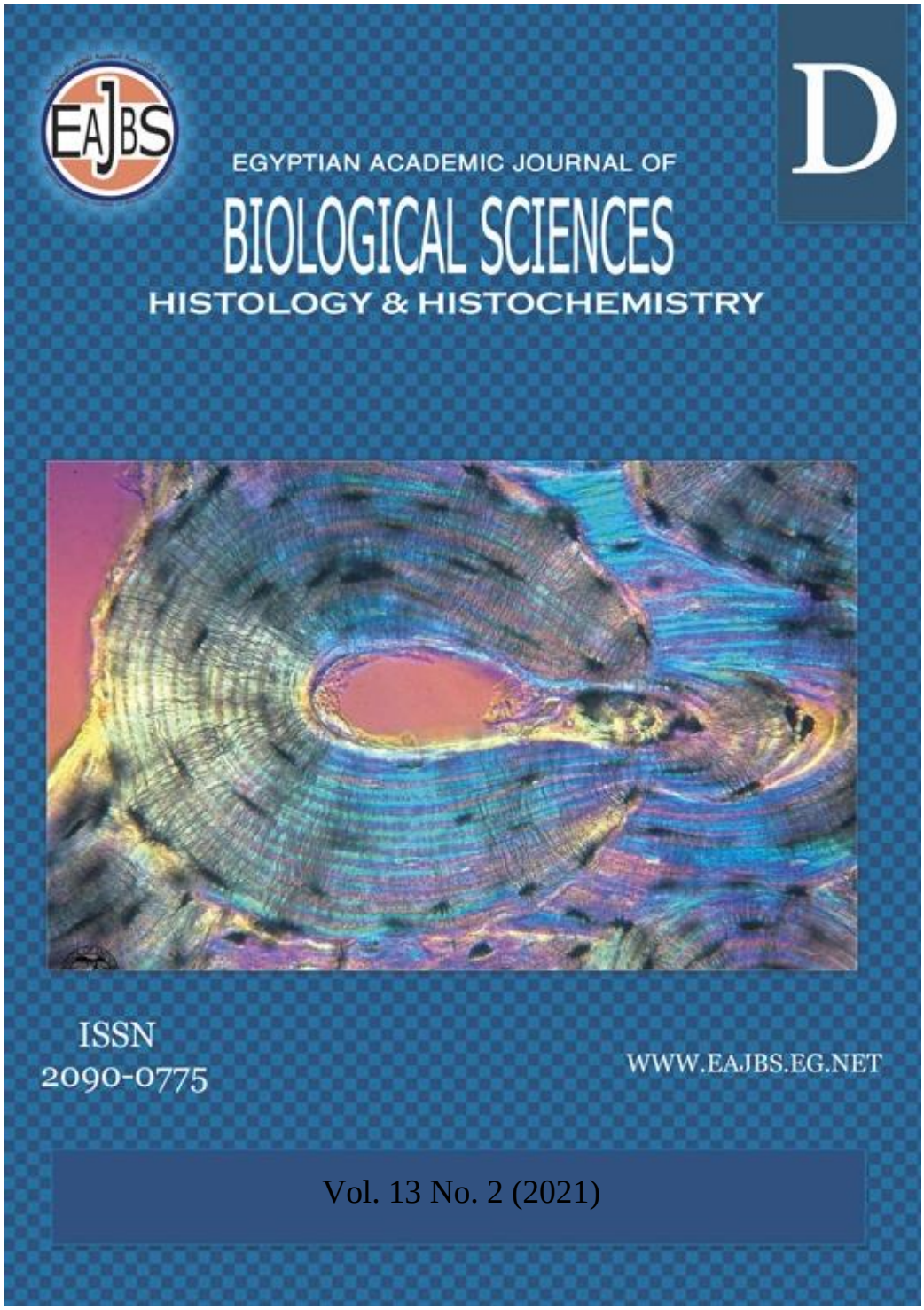

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Adipose-Derived Mesenchymal Stem Cells Partially Compensating the Neurodegenerative Signs at The Behavioral, Physiological, and Molecular Levels in AD Rat Model

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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia. Due to the multifaceted nature of AD pathology and limited understanding of its etiology, AD is difficult to be treated with currently available pharmaceuticals. Recent studies suggest that transplantation of mesenchymal stem cells might have therapeutic potential on several neurodegenerative disorders. In addition, it can ameliorate neuropathological deficits and physiological disorders in AD. The current study aims to evaluate the potential therapeutic effect of adipose-derived mesenchymal stem cells (ADMSCs) in AD rat model after the induction with $AlCl_3$.

After the induction phase, labeled ADMSCs have been injected intravenously. Open-field behavioral tests were conducted, serum β-amyloid levels, cholinesterase activity, and brain antioxidant status were evaluated. Besides, the expression of apoptotic and necrotic markers was quantified via RT-qPCR in brain tissues. Histopathological alterations in the brain were examined as well. Signs of dementia as manifested by behavioral tests have been recorded in AD rats, accumulation of β-amyloid in blood, reduced serum cholinesterase activity and both apoptotic and necrotic induction in brain tissues had been recorded at the end of the induction phase. All these alterations have been partially/fully compensated by the administration of ADMSCs, which proved their ability to penetrate the blood-brain barrier and home in the brain tissues. The molecular mechanism underlying the therapeutic effect of ADMSCs seems to be correlated with the reduction of neurodegeneration by upregulating the anti-apoptotic marker (Bcl2) and downregulating the pro-apoptotic markers (p53 and Bax) and necrotic factor (Tnfa) simultaneously.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of age-related dementia affecting more than 44 million individuals around the world and this number is expected to show an increase to more than 135 million individuals around the world by 2050 (Alzheimer's Association 2009; Albanese *et al.*, 2014).

AD is characterized by progressive memory loss which in return affects cognitive abilities, and this is associated with neural death (kim *et al.,* 2012). Well-known AD hallmarks are extracellular β-amyloid plaques accumulation, intracellular formation of neurofibrillary tangles (blurton-Jones *et al.,* 2009; Oddo *et al.,* 2003) and extensive inflammation (Meda *et al.,* 1995).

More recent research has pivoted focus to cytokine-mediated neuroinflammation as a major contributor to the development of AD, and among the cytokines involved in neuroinflammation, tumor necrosis factor α (TNF-α) is the most studied cytokine. Evidence suggests that inflammation promotes pathological processes that lead to AD (Akiyama *et al.,* 2000; Tarkowski *et al.,* 2003; McCaulley *et al.,* 2015), where many factors and signaling pathways activated by inflammation are involved in the regulation of cell apoptosis (Yang *et al.,* 2015). Aβ plaque formation induces neuronal apoptosis and contributes to the pathophysiology of AD (Simon *et al.,* 2011). Research has suggested that hyperphosphorylated tau, (Dias-Santagata *et al.,* 2007; Stamer *et al.,* 2002), inflammation (Candore *et al.,* 2010; Lee *et al.,* 2010) and β-amyloid (Aβ) accumulation (Zhao *et al.,* 2013; Yan *et al.,* 2013) are the basic mechanisms underlying the induction of oxidative stress and play a significant role in the pathogenesis of AD.

No drug therapy can reverse the inexorable neurodegeneration associated with AD, interrupt it or even slow it down (Small and Bullock 2011). Although anti-dementia therapies may slow the rate of progression of AD, pharmacotherapy for the behavioral and affective concomitants of AD is often non-rewarding and carries risks of potentially serious side effects. The available therapeutic drugs will only momentarily enhance cognitive abilities and slow the rate of progression of AD. On the other hand, non-drug therapies, including gene therapy and psychosocial-environmental treatments, offer only temporary symptomatic relief, and usually do not stop the progression of the disease (Tuszynski *et al.,* 1995; Rosenberg *et al.,* 1998; Wallbridge *et al.,* 2008, Manepalli *et al.,* 2009).

There is an enormous demand for effective therapies such as stem cell-based therapies to treat AD. Adipose-derived mesenchymal stem cells (ADMSCs) are multipotent, but known for their ability to differentiate into mesenchymal and non-mesenchymal lineages (Anghileri *et al.,* 2008), being easily accessible, having a high proliferation rate *in vitro* and the ability to undergo differentiation into multiple cell lineages (Manepalli *et al.,* 2009). ADMSCs are the most suitable stem cells source for clinical application due to the possibility of intravenous transplantation of ASCs that are known to show no immune rejection, no ethical debate nor tumorigenesis. Intravenous injection is the most convenient, simple, and safest method (Ra *et al.,* 2011). In this study, we highlight the therapeutic potential of adipose-derived mesenchymal stem cells (ADMSCs) for the treatment of AD in a rat model at the behavioral, physiological, and molecular levels.

MATERIALS AND METHODS

Experimental Animals, Feeding and Maintenance:

Thirty-two male albino rats (mean average weight 170 ± 10 g) were purchased from National Research Center. Animals were housed in the animal house, Zoology Department, Faculty of Science, Cairo University with 12 h/ 12 h light/dark cycle at (25±2°C) plastic cages with stainless cover (5 animals per cage), given standard pelleted diet (20% proteins) ad-libitum and get free access to tap water from plastic bottles. Animals were acclimatized to experimental conditions for 1 week. Experimental

protocols and procedures were approved by Cairo University, Institutional Animal Care and Use Committee (CU-IACUC) (Egypt) (CU/I/F/60/19), in accordance with international guidelines for the care and use of laboratory animals.

Experimental Design:

AD Induction: In Alzheimer's disease rat model (AD group), each rat was given an oral dose of $AlCl_3$. 5 H_2O (100 mg/ kg bw) dissolved in distilled water *very cautiously* daily for 5 weeks (Kumar *et al*,. 2009).

Mesenchymal Stem Cells: The MSCs were purchased from Prof. Dr. Laila Rashid (Kasr El Ainy, Giza, Egypt). After five weeks of AD induction, each rat in AD group was given a single dose of PKH26 green fluorescent-labeled ADMSCs ($1x10^6$ cells/0.5 ml DMEM per rat) (Lykhmus *et al.*, 2019) administrated intravenously in the tail vein, while the control group was given 0.5 ml of the vehicle (DMEM). The experimental work has been designed to include two successive phases: induction phase (I) for 30 days, and therapeutic phase for 35 days (T) as shown in table (1)

Serum Separation: After 5 weeks of the experimental period, 4 rats from each group were euthanized by cervical dislocation under anesthesia (100 mg sodium pentobarbital). Blood was collected from the retroorbital plexus according to (Van *et al.*, 2001). Serum was obtained and stored at −20 °C for subsequent biochemical assays.

Brain Sampling: Brains were excised, washed in physiological saline, dried on filter papers, and weighed. Brains were dissected into two halves, one was fixed in 10% formalin for histopathological examination and homing of different brain areas namely cerebral cortex, cerebellum, hippocampus, and medulla oblongata and the other was homogenized in 10% (w/v) phosphate buffer saline (PBS) and kept frozen at -80°C for subsequent molecular analyses.

Weight Measurements: The bodyweight of animals, as well as brain weights and brain somatic index, were recorded during the whole experiment**:**

Open-field Behavioral Tests: All behavioral tests were performed daily between 9 am and 11 am under standard conditions. Five standard open field tests, namely rearing, grooming, line center square, line crossing, and freezing were observed according to (Prut et al. 2003).

Estimation of Acetyl Cholinesterase (AChE) Activity: Serum AChE activity was assayed using spectrophotometric kit of Biodiagnostic, Dokki, Giza, Egypt following the instructions of the manufacturer. The absorbance change was measured using a kinetic method at 412 nm

Measurement of β-amyloid Peptide: Serum β-amyloid levels were measured by the ready-made ELISA kit supplied by (ANOVA, Keyuan Road, Daxing Industry Zone, Bljing, China) according to the manufacturer's instructions. The absorbance was measured at 450 nm using TECAN Infinite® 200 PRO microplate reader (Männedorf, Switzerland).

Assessment of Oxidative Stress Markers: Brain oxidative stress markers were detected using spectrophotometric ready-made kits of Biodiagnostic, Dokki, Giza, Egypt according to the method of (Ohkawa et al. 1979) for lipid peroxidation (MDA), (Aykaç et al. 1985) for reduced glutathione (GSH), (Kakkar et al. 2002) for superoxide dismutase (SOD) and (Aebi 1984) for catalase (CAT), respectively.

Quantitative Real Time-Polymerase Chain Reaction (RT-qPCR): Total RNA was extracted from brain homogenates of rat groups using GeneJET Viral DNA and RNA Purification kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol. After measuring its concentration using nanodrop (Thermo scientific, USA), 1 μg of the total RNA

was reverse transcribed into first-strand cDNA using Revert aid cDNA synthesis kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol (23). Then, RT- qPCR was performed using HERAPLUS RT- qPCR SYBR® Green master mix kit (Willowfort, Birmingham, UK), based on the manufacturer's protocol, to examine the expression of Tnfa gene, in addition to the pro-apoptotic marker genes; p53 and Bax, and finally the anti-apoptotic marker gene; Bcl2, and they were all normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (Gapdh) as a housekeeping control gene. Primers used for RT- qPCR were commercially synthesized from Macrogen, Inc. (Seoul, Korea), and their sequences are listed in Table 2. RT-qPCR was performed in applied Biosystems Step One Plus (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and each sample was prepared as a triplet for each gene. Each sample was initially denatured at 95 ℃ for 10 min, and then was subjected to 40 cycles of denaturation at 95 ℃ for 15 sec, annealing and extension at 60 ℃ for 1 min, then final extension at 72 ℃ for 10 min and finally held at 4 ℃ followed by amplification and melting curves to ensure reaction specificity. Following RT-qPCR, Ct values were measured, ΔΔCt and fold expression were calculated to quantify the results (Livak and Schmittgen, 2001).

Homing of ADMSCS in Brain Tissues: One rat from ADST group was injected with a single dose of labeled 1 x 106 ADMSCs intravenously through the tail vein. *In Vivo* Fluorescence measurements were performed 2 days post-injection and fluorescence images were obtained using (Olympus microscope) for data analysis.

Histopathological Examinations:

Fixed brain tissues have been processed by ordinary routine work: dehydration, clearing, and embedding. The paraffin-embedded blocks of the different specimens were cut by using microtome in 4 µm thick tissue sections and stained by hematoxylin and eosin (H&E) for exposing histopathological examinations.

Statistical Analysis:

Values were expressed as (mean ± SE). Statistical analysis was performed using SPSS statistical software package version 25 by one-way analysis of variance (ANOVA) with Duncan post hoc test and $P \leq 0.05$ was considered statistically significant.

RESULTS

1. Changes in Bodyweight, Brain Weight and Brain Somatic Index:

As shown in fig. (1a), AD rats exhibited a gradual and significant reduction in body weight during the induction phase as compared to control rats (CI). As we proceed to the therapeutic phase, ADST group showed a slight and gradual increase at the early stages of the therapeutic phase, but a remarkable and significant increment as compared to AD rats almost reaching the control values by the end of the therapeutic phase. In opposition, the untreated group failed to achieve a remarkable increase in weight even after the withdrawal of $AlCl_3$.

Data presented in figures (1b and 1c) showed insignificant changes in brain weight between groups in the two successive experimental phases. As regards brain somatic index, data exhibited an insignificant change in AD group versus CI group during the induction phase, but a remarkable and insignificant reduction in ADT group versus control and stem cell-treated groups (24.8% and 19.27% respectively) even that $AlCl_3$ has been withdrawn.

2. Open Field Behavioral Tests:

As illustrated in figure (2), the present data showed a gradual and significant decrease in (rearing, grooming, line crossing and center square crossing) simultaneously with a significant increase in freezing indicating some signs of depression and dementia as signs of AD. *A single*

administration of ADMSCs has shown to partially compensate for these behavioral disorders especially in (rearing, grooming, freezing and line crossing).

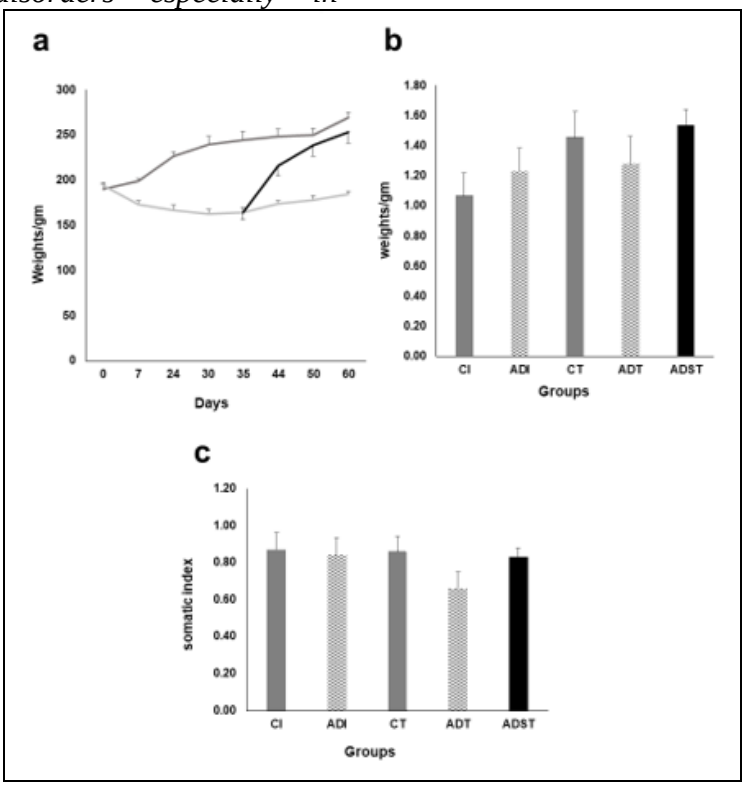

Fig.1: Change in rat **a)** body weights **b)** brain weights **c)** brain somatic index throughout the entire experimental period ($AlCl_3$ induction phase and single administration of ADMSCs therapeutic phase) on the Alzheimer's disease rat model

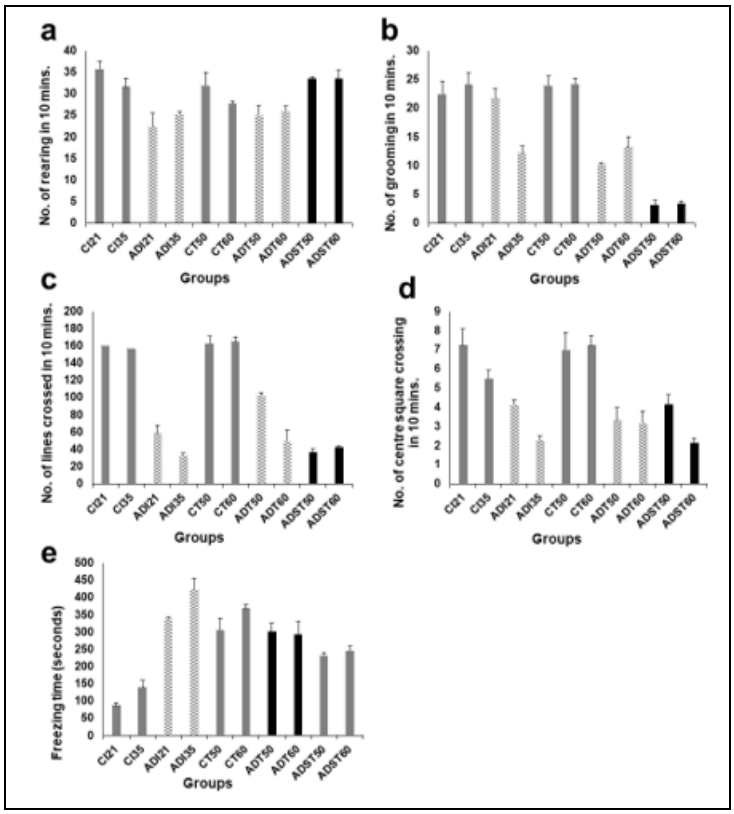

Fig. 2: Changes in the number of **a)** rearing **b)** grooming **c)** line crossings **d)** center square crossings and the time of **e)** freezing throughout the entire experimental period on the Alzheimer's disease rat model.

During the induction phase, AD rats (ADI) showed a significant increase in the concentration of ACHE compared to the control group (CI) *indicating signs of depression and disturbances in the transmission of nerve impulses in the brain*. The untreated AD rats (ADT) still exhibited a significant increase in the concentration of ACHE compared to the control group (CT) and ADI group while the administration of a single dose of ADMSCs elicited a significant decline in serum ACHE compared to the ADI group and control group (CT) (Fig.3).

4. β-amyloid Measurement:

As demonstrated in figure (3), AD rats showed a non-significant change in serum β-amyloid at the end of the induction phase, but it showed a significant increase in the middle of the therapeutic phase. This indicates the late onset of accumulation of β-amyloid plaques after withdrawal of aluminum chloride as a sign of dementia. Regarding the therapeutic phase, the untreated AD rats (ADT) showed a significant increase of (Aβ), while the stem cells administrated group showed a non-significant decrease of (Aβ).

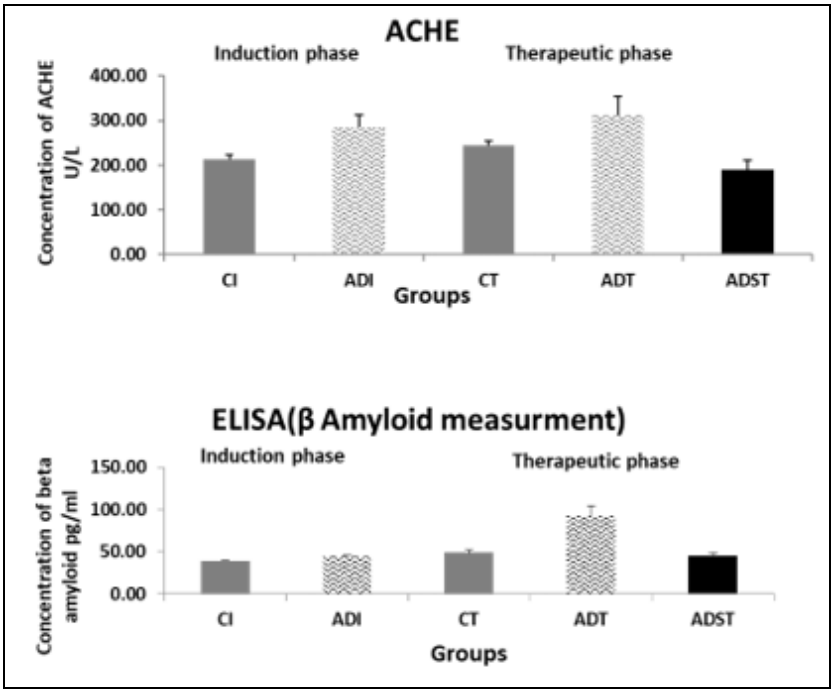

Fig.3: Change in **a)** ACHE concentrations **b)** beta amyloid concentrations throughout the entire experimental period ($AlCl_3$ induction phase and single administration of ADMSCs therapeutic phase) on the Alzheimer's disease rat model.

5. Oxidative Stress Markers:

During the induction phase, ADI group showed a significant increase in the oxidative stress marker (MDA). Regarding the antioxidant molecules, the CAT showed a significant increase in its concentration while the GSH and SOD showed a significant decrease in their concentrations, compared to the CI group. The untreated AD rats (ADT) showed a significant increase in the MDA and CAT concentrations and no significant change in the GSH, and SOD concentrations compared to the AD group. But during the therapeutic phase, the ADST group showed a significant decrease in MDA. Meanwhile, the GSH showed a significant decrease in its concentrations, a significant increase in CAT concentration and no significant change in the SOD concentration, compared to the ADI group (Fig. 4).

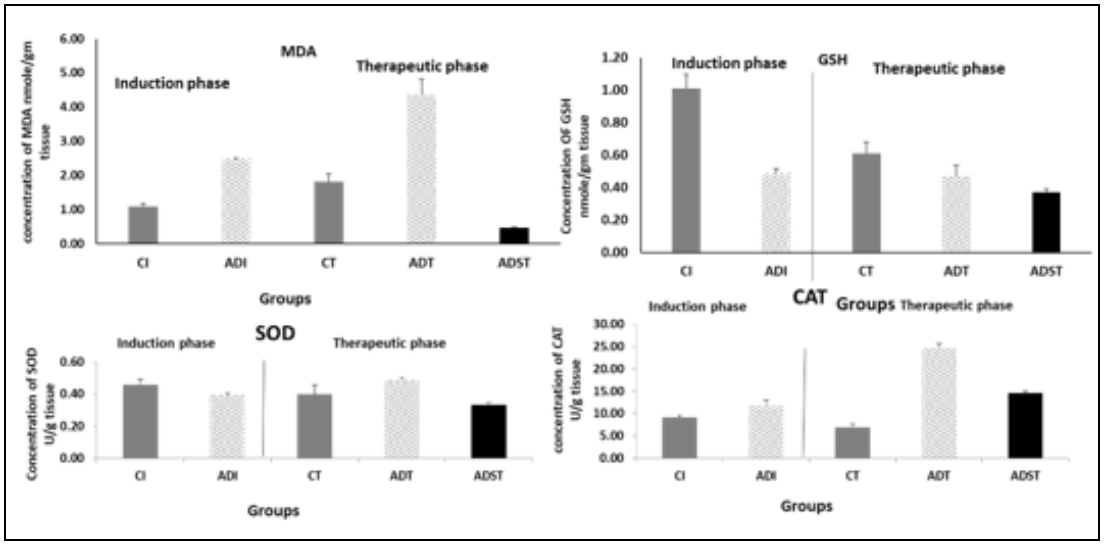

Fig.4: Change in concentrations of **a)** MDA **b)** GSH **c)** SOD and **d)** CAT throughout the entire experimental period ($AlCl_3$ induction phase and single administration of ADMSCs therapeutic phase) on the Alzheimer's disease rat model.

6. Expression of Pro-Apoptotic, Anti-Apoptotic and Necrotic Genes In Brain AD Rats:

As demonstrated in figure (5), AD rats showed a significant increase in fold expression of pro-apoptotic markers (p53 and Bax) compared to the control group during the induction phase. It is also clear that there is a gradual decrease in fold expression in the proapoptotic genes in the ADT group, *which might indicate the aluminum withdrawal effect*. While in the therapeutic phase, the three groups showed a significant decrease back to normal in fold expression. On contrary, the ADI group showed a significant decrease in fold expression of Bcl2 versus the control during the induction phase. Meanwhile, in the therapeutic phase, there was a much more significant decrease in fold expression of Bcl2 in the ADT group, but there was a remarkable significant increase in fold expression of Bcl2 in the stem cell-treated group.

Regarding necrosis, comparing the AD rats with the control mates there was a significant increase in the fold expression of Tnfa in the induction phase. There is a gradual insignificant decrease in the fold expression of Tnfa in the ADT group. Meanwhile, there was a gradual significant decrease in the fold of expression of Tnfa in the stem cell-treated group in the therapeutic phase.

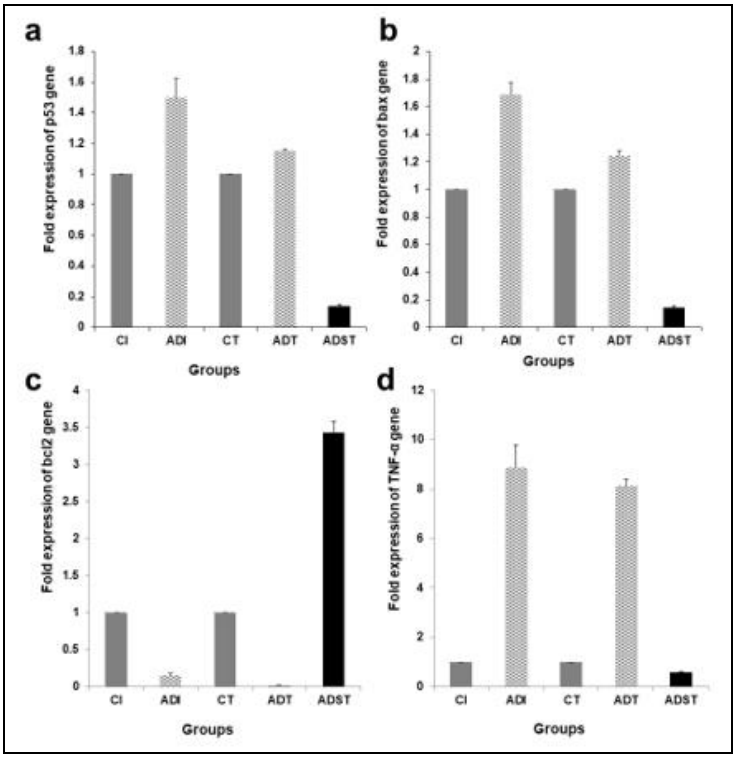

Fig.5: Fold change in expression of pro-apoptotic markers **a)** p53 **b)** Bax, anti-apoptotic marker **c)** Bcl2 and **d)** tumor necrosis factor-a (TNF-a) CAT throughout the entire experimental period ($AlCl_3$ induction phase and single administration of ADMSCs therapeutic phase) on the Alzheimer's disease rat model.

7. Homing Test:

One of the merits of ADMSCs is their ability to home at the site of injury. The present study has shown this spectacular movement of stem cells to the brain tissue *indicating their potential to cross the blood-brain barrier* as demonstrated in figure (6).

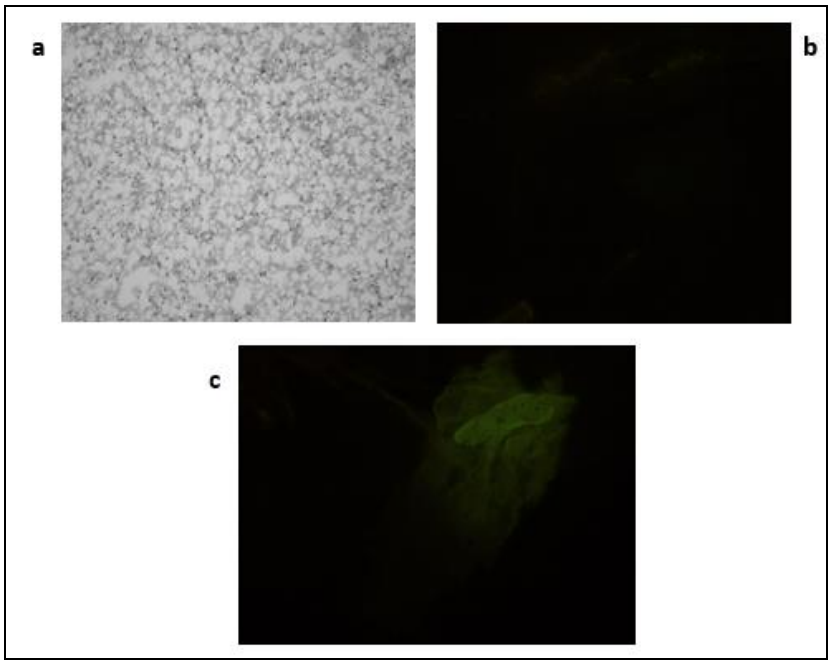

Fig.6: Photomicrograph in (a) unstained brain tissue by bright field microscope (b) control group by fluorescent microscope (c) ADMSCs in the brain of AD rat model 2 days post ADMSCs injection by fluorescent microscope for showing homing.

8. Histopathology:

As seen in photomicrographs **(7),** the present study demonstrated signs of neurotoxicity as neuronal degeneration, focal amyloid plaques, and gliosis with hemorrhage around cerebral blood vessels in the cerebral cortex in ADI rats compared to the control ones. A single administration of ADMSCs *has shown partial improvement in the neurodegenerative signs of AD in brain tissues compared to the control rats.*

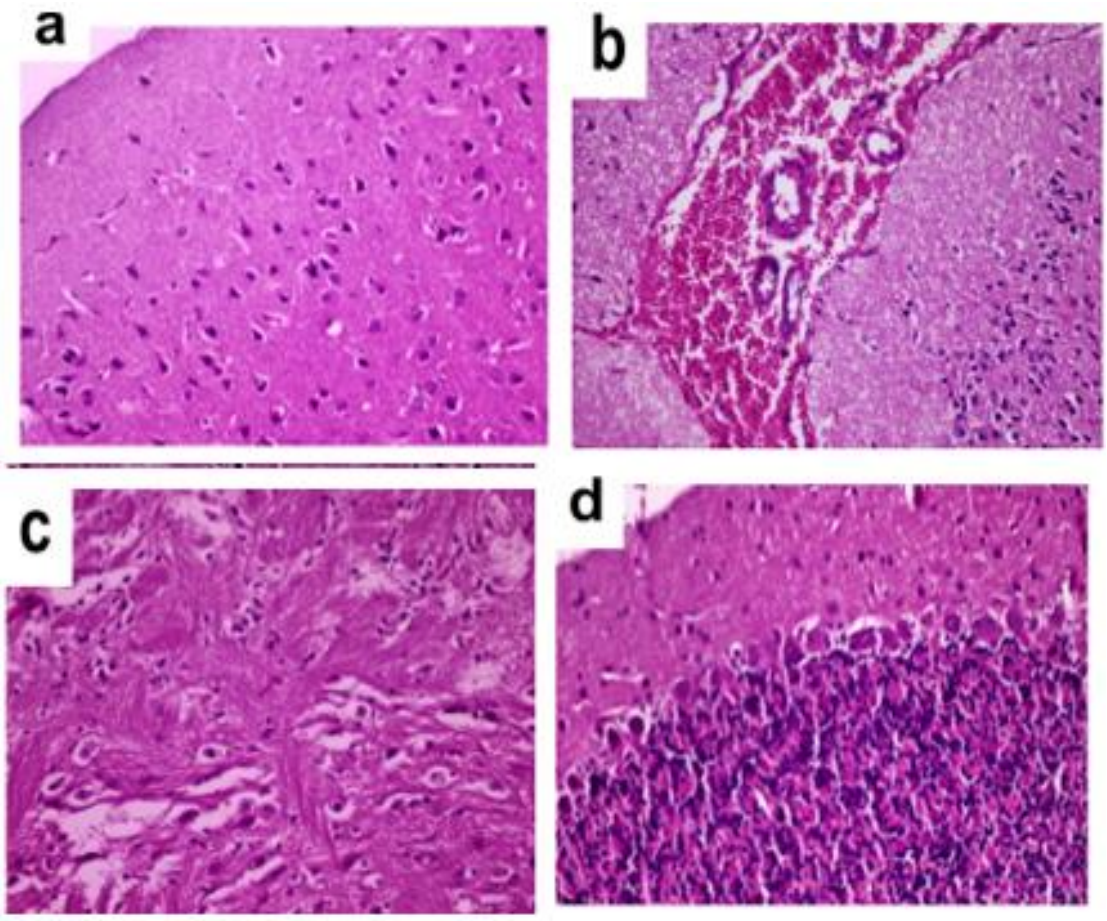

Fig.7: Sections taken from the brains of **(a)** control rats at the end of the induction phase showing normal histological structures stained by Hematoxylin. Eosin stain of cerebral cortex (X400), **(b)** rats given aluminum chloride at a dose of (100 mg/kg) at the end of the induction Phase showing cerebral cortex gliosis with hemorrhage around cerebral blood vessels (H&E X400), **(c)** rats treated with ADMSCs after 9 days of therapeutic phase showing Cerebral Striatum neuronal degeneration and gliosis (H&E X400), **(d)** rats given aluminum chloride intravenously as a single dose of (1 x 10^6 cells) after 25 days of therapeutic phase and 60 days that Cerebellum showing apparent normal Purkinje cells (H&E X400)

DISCUSSION

The present study discussed that Alzheimer's disease (AD) is a progressive neurodegenerative disease and is the most common form of dementia. Despite the high prevalence of AD, there are still no treatments to stop or reverse its progression, but only temporarily improve its symptoms. Therefore, the present study aimed to evaluate the therapeutic potential of adipose-derived mesenchymal stem cells (ADMSCs) in AD rat model after the induction by $AlCl_3$.

The present study was successful in experimental induction of AD in a rat model by injection of $AlCl_3$ in the dose used. This was clear from

the signs of dementia, open field behavioral tests especially rearing, grooming, and freezing accompanied by loss of appetite and reduction in body weight. In addition, these signs were also supported at the physiological level as manifested by an increase in AChE activity, gradual accumulation of β-amyloid, increase in oxidative stress marker (MDA) and decrease in the antioxidant defense system. At the molecular level, there was an increase in apoptotic and necrotic markers which was verified by signs of degeneration in different brain areas at the histopathological level.

The present study showed a gradual and significant reduction in body weight in AD rats compared to the control ones during the induction phase. This seems to be due to the neurotoxic effect of $AlCl_3$ which caused loss of appetite as a sign of neurodegeneration. After a single injection of ADMSCs, the values of body weight remarkably and significantly increased almost reaching the control values by the end of the therapeutic phase. This reflects the therapeutic potential of a single dose of ADMSCs in improving the appetite of rats, which is consistent with the report of (Thenmozhi *et al.,* 2015).

As concerns brain weight, the present data showed insignificant changes among different groups in the two successive experimental phases. Moreover, data showed insignificant changes in brain somatic index in AD group versus CI group during the induction phase, but a remarkable and insignificant decline in ADT group as compared to CT and ADTS groups even after $AlCl_3$ has been withdrawn. The present results might provide additional evidence to the fact that Alzheimer is a progressive disease (Joseph *et al.,* 2009; Schmidt *et al.,* 2011; Lane *et al.,* 2017; Cenini *et al.,* 2019; De Ture *et al.,* 2019).

The present study has also documented the signs of neurodegeneration in the $AlCl_3$-induced AD rat model in terms of anxiety, locomotion, activity and behavior as supported by (Abdel-Wahab 2012). These signs seem to be correlated with the accumulation of aluminum chloride in the rat brain as postulated by (Thenmozhi *et al.,* 2015). In coincidence with the present data, (Thenmozhi *et al.,* 2015; Rashwan *et al.,* 2018) have reported a significant decrease in rearing and grooming in $AlCl_3$-induced AD rats. On the other hand, (Rashwan *et al.,* 2018) showed a significant increase in time of freezing in $AlCl_3$-induced AD rats supporting the present finding. After a single administration of ADMSCs, the preset data have shown signs of partial compensation of the above-mentioned behavioral disorders especially in rearing, grooming, freezing as well as line crossing. This partial compensation might be correlated with the duration of the therapeutic phase and also the individual behavioral variation.

Acetylcholine esterase (ACHE) is a key enzyme that functions to terminate the effect of acetylcholine released by the motor neurons. In the present study, the activity of serum ACHE has demonstrated a significant increment in AD group at the end of the induction phase as compared to control rats, which has been compensated by the single administration of ADMSCs. These findings run in parallel with those claimed by (García-Ayllón *et al.,* 2011) and reflect the potential role of ADMSCs in restoring the motor activity in the brain.

One of the main causes of AD is the accumulation of the β-amyloid plaques in the brain disrupting the signaling process causing brain dysfunction. In the present study, AD rats showed a gradual increment of serum β-amyloid concentration with late-onset of accumulation of β-amyloid plaques after withdrawal of aluminum chloride as a sign of dementia. These findings have also been documented by the histopathological findings in the present study demonstrating the accumulation of focal amyloid plaques

in the cerebral cortex and hippocampus. Once again, this proves that Alzheimer is a progressive disease. Under the present experimental condition, the single administration of ADMSCs has succeeded to significantly decrease β-amyloid plaques at the end of the therapeutic phase versus ADT group. These findings ran in concomitance with those previously reported by (Kim *et al.,* 2012) who proved the ability of multiple administration of ADMSCs to dramatically reduce serum β-amyloid plaques after 4 months in AD-mice model.

Oxidative stress is known as an imbalance between oxidants and antioxidants favoring the oxidants and it plays a significant role in the pathogenesis of AD. Malondialdehyde (MDA) is commonly known as an oxidative stress marker. The present study showed a significant increase in brain MDA in AD group versus the control one during the induction and therapeutic phases. This observation has also been documented in previous reports by (Skoumalová *et al.,* 2012). On the other side, CAT, GSH and SOD are among the antioxidant defense system that scavenges the free radicals and quenches the oxidative stress. In the present study, brain CAT activity showed a significant increase in ADI rats versus the controls, which disagrees with the previous report by (Wojsiat *et al.,* 2018), while brain GSH and SOD showed a significant decrease, which agrees with the report of (Wojsiat *et al.,* 2018). These findings could be discussed in view of the neurodegenerative phenomena of AD with a massive reduction number of active defending nerve cells. In addition, the accumulation of excess free radicals stimulates more consumption of antioxidants resulting in the depletion of the body's antioxidant reserve as reported by (Padurariu *et al.,* 2013). The present investigation has proved the potential activity of ADMSCs to counteract the adverse effects of Al toxicity on the oxidative balance in brain tissues. This observation has been documented in ADST versus CT and ADT during the therapeutic phase. These findings are recently supported by the work of YuanBo *et al.* (2017), Stavely and Nurgali (2020), Angeloni, *et al.* (2020) as regards brain MDA and CAT but disagree with those claimed by Angeloni *et al.* (2020) as regards GSH and SOD.

AD is a neurodegenerative disease that seems to be due to elicited apoptosis and necrosis (Shimohama 2000; Behl C 2000; Obulesu *et al.,* 2014; Chang *et al.,* 2017; Tanaka *et al.,* 2020). Hence, the present study assessed the mRNA expression of pro-apoptotic markers (Bax and p53), anti-apoptotic marker (Bcl2) and Tnfa genes to prove this hypothesis in terms of quantitative gene expression. The present data give support to this assumption by demonstrating significantly upregulated p53, Bax and Tnfa and downregulated Bcl2 in AD group as compared to control one during the induction phase. These findings are supported by the previous reports of Joseph *et al.,* (1997), Liu *et al.* (2010). Fortunately, the present study has shown that a single injection of ADMSCs was able to counteract these changes back to normal values. Therefore, the intravenous injection of mesenchymal stem cells led to a significant improvement of memory deficits in AD rat models via the suppression of apoptosis and necrosis. The present study has additionally supported the claims of (Kim *et al.,* 2012) that ADMSCs are able to cross the blood-brain barrier and home at the site of injury (brain).

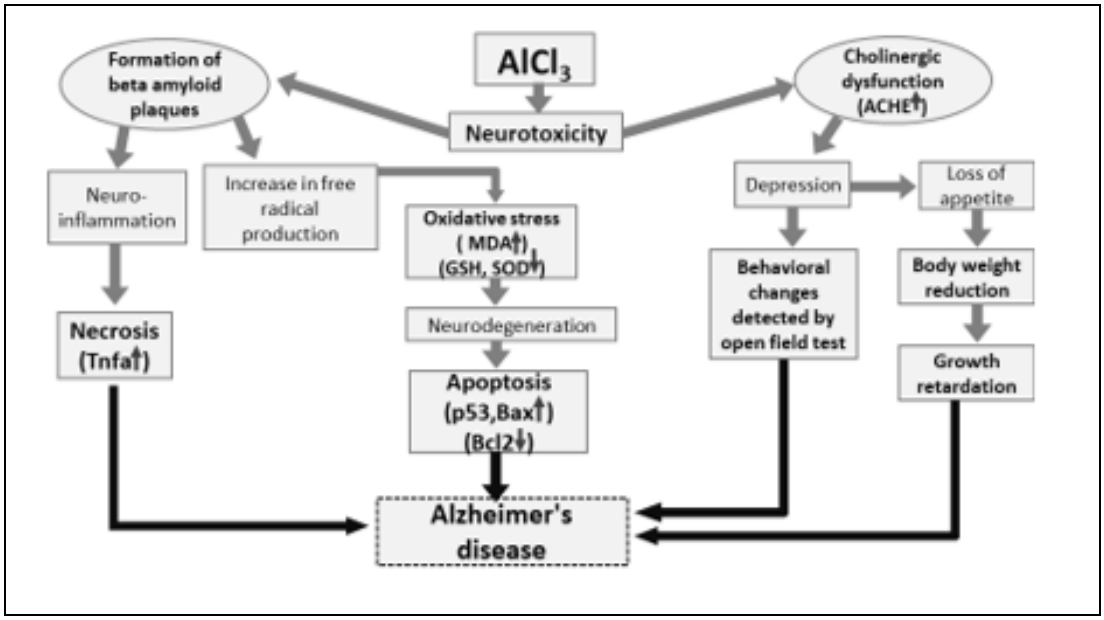

Fig. 8- Schematic representation of Alzheimer's disease signs after $AlCl_3$ induction in rat model

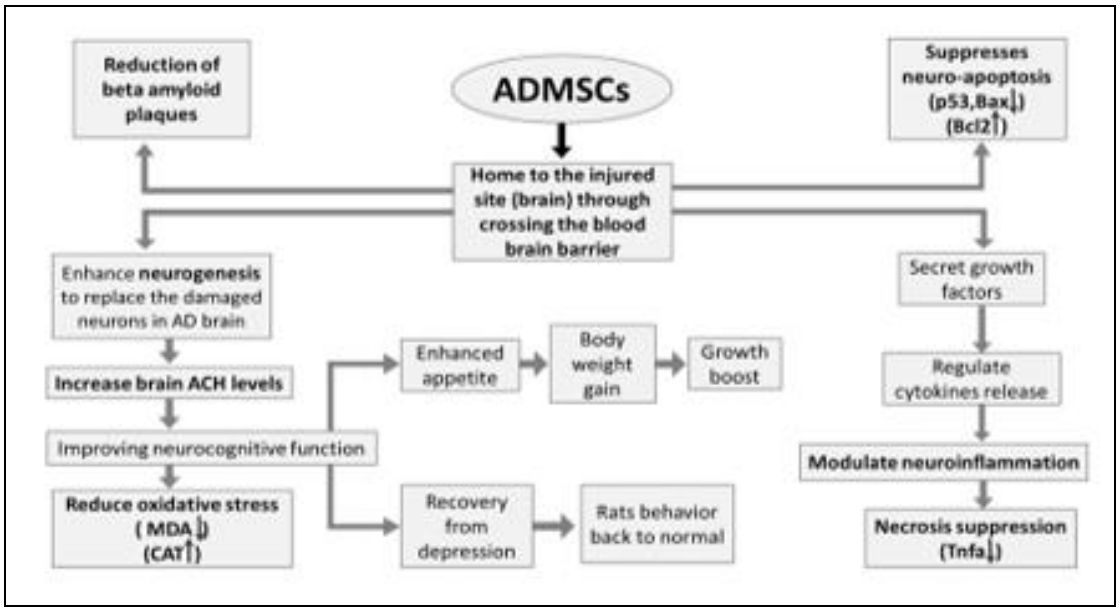

Fig. 9- Schematic representation of the compensatory effect of a single administration of ADMSCs in rat model.

CONCLUSION

As illustrated in Figures (8 and 9), the present study was successful in experimental induction of AD in a rat model by injection of $AlCl_3$ in the dose used. This was clear from the signs of dementia, open field behavioral tests especially rearing, grooming, and freezing accompanied by loss of appetite and reduction in body weight. In addition, these signs were also supported at the physiological level as manifested by an increase in AChE activity, gradual accumulation of β-amyloid, increase in oxidative stress marker (MDA) and decrease in the antioxidant defense system. At the molecular level, there was an increase in apoptotic and necrotic markers accompanied by signs of degeneration in different brain areas at the histopathological level. The present study has also demonstrated that injection of a single dose of ADMSCs improved the appetite of AD rats, which in turn improved their body weights, partially compensated for the behavioral disorders associated with Al

neurotoxicity, and restoring the motor activity in the brain. Furthermore, the single administration of ADMSCs decreased β-amyloid plaques at the end of the therapeutic phase and counteracted the adverse effects of Al toxicity on the oxidative balance in brain tissues. In addition, the intervention with ADMSCs had led to a significant improvement of memory deficits in AD rats via the suppression of apoptosis and necrosis. Ultimately, the present data has illustrated the therapeutic potential of ADMSCs for compensation of many symptoms of AD in a rat model at behavioral, physiological, molecular, and histopathological levels under experimental conditions.

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Authors' contributions: Emad M Elzayat and Mohamed Hosney have conceived, designed, planned, and supervised the research point. All authors performed the experimental work. Emad M Elzayat and Mohamed Hosney analyzed and interpreted the data. Yasmine Elbeltagy, Asmaa Husein and Fatma Mohamed were major contributors in writing the manuscript. All authors participated in summarizing the findings in the form of a schematic diagram, reviewed, and edited the final version of the manuscript.

Compliance with Ethical Standards:

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ARABIC SUMMARY

التعويض الجزئى للخلايا الجذعية الوسيطة الدهنية لعلامات التلف العصبي في مراحلالسلوكي والفسيولوجي والجزيئي في نموذج الفئران المصابة بالزهايمر

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يعتبر مرض الزهايمر (AD) هو الشكل الأكثر شيوعًا من الخرف الذي يحدث في الغالب بعد 65 عامًا. تتميز التغيرات المرضية العصبية الرئيسية في مرض التهاب الدماغ العصبي المزمن وفقدان الخلايا العصبية بسبب التشابك الليفي العصبي (NFTs) من تاو المفرط الفسفورية بشكل غير طبيعي، لويحات am- الأميلويد (Aβ) واختلال وظائف التمثيل الغذائي المختلفة. نظرًا للطبيعة متعددة الجوانب لأمراض مرض الزهايمر وفهمها المحدود لمسببات المرض، يصعب علاج مرض الزهايمر باستخدام الأدوية المتاحة حاليًا. تشير الدراسات الحديثة إلى أن زرع الخلايا الجذعية الوسيطة قد يكون له إمكانات علاجية لعدة اضطرابات تنكسية عصبية، بالإضافة إلى أنه يمكن أن يخفف فعليًا حالات العجز المرضية العصبية والاضطرابات الفسيولوجية في مرض الزهايمر. تهدف الدراسة الحالية إلى تقدير التأثير العلاجي المحتمل للخلايا الجذعية الوسيطة المشتقة من الشحوم (ADMSCs) على مرض الزهايمر في نموذج الفئران بعد تحريض المرض باستخدام كلوريد الألومنيوم.

تم إحداث AD بواسطة 100) $AlCl_3$ ملغم / كغم من وزن الجسم) المعطى عن طريق الفم لمدة 5 أسابيع. بعد تحريض AD، تم حقن PKH26 ADMSC ذو العلامات الفلورية الحمراء عن طريق الوريد (106 خلية / فأر، معلق في وسط DMEM) في الوريد الذيل. تم إعطاء الفئران المجموعة الضابطة DMEM فقط. الموت الرحيم للجرذان عن طريق الخلع عنق الرحم بعد أخذ التخدير (B100 k / mg. بالوزن الصوديوم بنتوباربيتال). بعد تشريح، تم الحصول على الدم والأنسجة، تم فصل المصل والحفاظ على تجميدها في -80 درجة مئوية لقياس الجزيئي. تم الحفاظ على الأنسجة في الفورمالين 10 ٪ لفحص التشريح المرضي وصاروخ موجه. تم إجراء سجل إجمالي للوزن واختبارات سلوكية مفتوحة، وتم قياس مستويات الأميلويد في المصل وأنسجة المخ بواسطة ELISA. بالإضافة إلى ذلك، تم قياس التعبير مرنا من علامات موت الخلايا المبرمج ، (P53 and Tnf α ، Bax و Bcl2) بواسطة RT-qPCR في أنسجة المخ. تم فحص التغيرات المرضية في الكبد والكلى والرئة والقلب والطحال والخصية. أظهرت الفئران فقدان الشهية وفقدان الوزن الإجمالي مقابل المجموعة الضابطة. أثبتت الاختبارات *السلوكية* المفتوحة المجال وجود خرف في الفئران التي عولجت $AlCl_3$ والتي تم تعويضها بعد علاج ADMSC. أظهرت مستويات المصل لـ am-amyloid مستويات أعلى في الفئران AD مقارنةً بالسيطرة على واحدة في نهاية النموذج التجريبي.