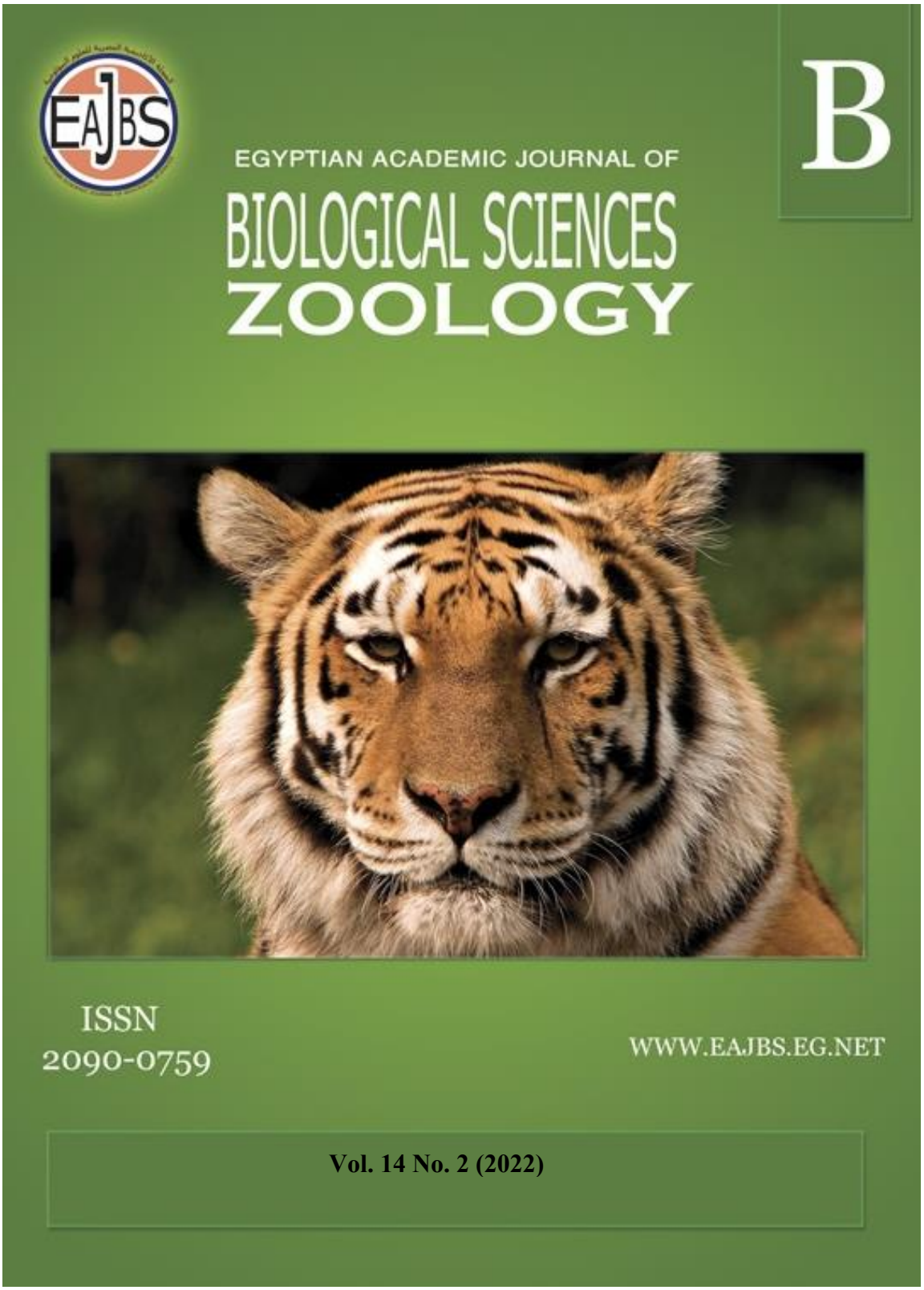

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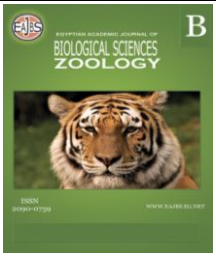

Therapeutic Potential of Adipose-Derived Mesenchymal Stem Cells (ADMSCs) with/without Taurine in Aluminum Chloride-Induced Alzheimer's Disease Rat Model

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ABSTRACT

Alzheimer's disease (AD) is the most type of dementia characterized by its progression, neurobehavioral and neuro-pathological characteristics. Adipose-derived mesenchymal stem cells (ADMSCs) have previously proved a potential role in preventing the pathogenesis of several neurodegenerative disorders, so it is regarded as a promising new approach for AD regenerative therapy. Taurine was found to enhance stem cell activation and propagation, yielding a higher concentration of neural progenitors and stem cells, and reducing the number of activated microglia leading to down-regulated inflammation *in vitro*. The present study aimed to investigate the possible therapeutic potential of ADMSCs with/without taurine in treating the AD rat model. It was planned to include three successive phases: induction, withdrawal, and therapeutic phases. Fifty male Wistar rats were divided into two main groups: control (C) and AD model. Behavioral changes, as manifested by the T-maze experiment, had been recorded. β-amyloid levels had been measured in brain homogenate and serum by ELISA. Oxidative stress marker (MDA), brain and serum antioxidant enzyme activities (SOD, GSH, and CAT) as well serum acetylcholine esterase activity were spectrophotometrically determined. Pro-apoptotic (p53 and Bax) and anti-apoptotic (Bcl2) gene expression in the brain were evaluated using RT-qPCR. The histopathological alterations in brain tissues were also observed.

INTRODUCTION

Alzheimer's disease (AD) is the most prevalent type of dementia; it is related to age, sex, apolipoprotein E genotype (ApoE), and amyloid precursor protein (APP) degradation products (Xiao *et al.,* 2017). AD is characterized by its progression, neurobehavioral and neuropathological alternations, the diverse neuronal loss throughout the basal forebrain, hippocampus, amygdala, and cortical area (Xiao *et al.,* 2017). Neurobehavioral alterations, including short-term memory loss in the early stage, are manifested by confusion, aggression, mood swings, long-term memory loss, and social

isolation in advanced stages, causing social and economic issues to public health disturbance (Thenmozhi *et al.,* 2015). Neurotransmitters have special neuroprotective effects versus dementia. There are numerous associated abnormalities in various neurotransmission systems in AD, like as noradrenergic, cholinergic, and dopaminergic systems (Aliev *et al.,* 2019). In 2015, about 47 million people with AD predicted that this number would reach about 131 million by 2050 (Prince 2015).

Pathologically, AD has two major hallmarks: insoluble extracellular β-amyloid AB accumulation in senile plaques and intracellular tau-protein filaments in the form of neurofibrillary tangles NTFs (Koo *et al.,* 2013). The pathological amyloid peptide oligomer is produced from amyloid precursor protein (APP), a transmembrane protein after a sequence of proteolytic effects of b c-secretases (Thenmozhi *et al.,* 2015). AD can be defined as irreversible cognitive function deterioration based on forebrain neuron loss of the cholinergic projection system in the nucleus basalis of Meynert (nbM) mainly due to extracellular accumulation of insoluble Aβ protein. Aβ is known as the common noxious element in the brain tissue of patients with AD. It was recognized that the accumulation of Aβ was able to AD development and participated in extensive neuronal impairment and cell necrosis (Eftekharzadeh *et al.,* 2020).

There are no consent measures to diagnose and regulate AD development (Cure et al., 2014), which significantly obstructs effective treatments for AD. To examine AD pathologies and their targets, various animal models of AD have been used in preclinical settings. The use of animal models has enabled scientists to investigate many diseases on different levels including behavioral, physiological, cellular, and molecular mechanisms of Alzheimer's progression (Jour *et al.,* 2011). In addition, animal models could be used for some tests that could be done only on animals rather than humans, to evaluate the primary effect of different agents including protective and therapeutic (Esquerda *et al.,* 2017). Among these, mice and rats are extensively used, and their transgenic complements are the most-established system to assess disease pathophysiology in addition to successful treatment strategies (Bali *et al.,* 2017). Rats are the most commonly used animal models due to their large size and body dimensions in addition to manipulation easily (Esquerda *et al.,* 2017).

Aluminum (Al) has a vital role in AD pathogenesis and etiology as a neurotoxic metal (Cao *et al.,* 2017), depending on clinical studies and many documented *in vitro* and *in vivo* (Thenmozhi et al., 2015). Previous studies showed that Al accelerates extracellular AB oligomers generation and deposition (Cao *et al.,* 2017; Thenmozhi *et al.,* 2015). Al is also acting as a cholino-toxin affecting the cholinergic activity, the main hallmark of AD neurochemistry (Thenmozhi *et al.,* 2015). Aluminum reaches the brain through the blood-brain barrier (BBB) via different routes such as diet, cosmetics, medications, toothpaste, drinking water and fumes inhalation. This led to its deposition in the cortex, cerebellum and hippocampus causing memory deterioration and triggering cognitive decline. In another word, AD was induced via AB accumulation after oligomerization and tau phosphorylation and aggregation in addition to apoptosis, impaired calcium ion exchange, and lipid peroxidation (Thenmozhi *et al.,* 2015).

Aluminum was reported in clinical trials to present in extremely higher levels in sporadic and familial AD brain tissues accounting for 10 pg/g tissue dry weight in 5/12 patients, compared to normal ones. Additionally, it is believed that AL can initiate an AD-like inflammation, as it induces the formation of fibrillary depositions. This formation is almost similar to neurofibrillary tangles, causing an adverse effect on memory and recognition abilities, that ultimately lead to the accumulation of oxidative stress. Therefore, it was used to create a model which mimics the AD brain, $AlCl_3$ which is a common form of Al (a major risk factor of AD). Moreover, the Al-induced rat model

of AD is an established model widely used to investigate the etiology and treatment strategies of the disease (Cao et al., 2017). To evaluate cognitive impairments in animal models, some behavioral tests were adopted including T-maze tasks, due to their sensitivity to memory deficits and hippocampal-dependent learning (Lee *et al.,* 2018).

The recently used treatments for AD have limited curative abilities, so finding a cure is a critical challenge that faces scientific research. Therefore, extensive effort has been enthusiastic into the development of new drugs that decrease the amyloid burden and slow down disease progression; however, effective therapeutic treatments have not yet been elucidated (Oh *et al.,* 2020). Stem cell therapy in regenerative medicine is one of the promising approaches aiming to help in treating neurodegenerative diseases especially AD (Kim *et al.,* 2012). Stem cells secret various essential components including growth elements and extracellular vesicles. There is no doubt that selecting the best source of stem cells has a vital role in transplantation therapeutic efficiency. Mesenchymal stem cells (MSCs) have anti-apoptotic, angiogenic, and supportive (induction of mitosis or proliferation) that contributed to the secretion of trophic paracrine factors (Eftekharzadeh *et al.,* 2020). Among the various adult MSCs, adipose-derived stem cells (ADSCs) are a feasible and appropriate basis in clinical studies. These stem cells are a suitable alternative for the therapy of neurodegenerative disorders since they can pass through the blood-brain barrier (BBB) and home to the injured parts of the brain. They also lack any tumor genesis, ethical issues, and immune rejection problems (Eftekharzadeh *et al.,* 2020). Based on the previous study, transplantation of ADSCs increased neurogenic effects in the subventricular zone and decreased oxidative stress that led to cognitive impairment (Yan *et al.,* 2014).

Taurine, 2-aminoethanesulfonic acid, is the second most common endogenous amino acid after glutamate in the central nervous system (CNC) (Albrecht et al., 2005). It plays vital roles in the body, including stabilization of protein folding, thermoregulation, antioxidation, anti-inflammation, calcium homeostasis, osmoregulation, and CNS development (Oh *et al.,* 2020). However, it is not incorporated in any protein synthesis, it is a key element in many physiological processes such as osmoregulation and bile acid conjugation, pharmacological actions, pathological states, and prevention of oxidant-induced injury in many tissues (Yildirim *et al.,* 2011). In biological systems, Taurine acts as an antioxidant via stabilizing bio-membranes, scavenging ROS, and reducing the peroxidation of unsaturated membrane lipids (Yildirim *et al.,* 2011).

Taurine is suggested to be a key element in neurotransmission, neuromodulation, and cell excitability. Taurine also has the ability to pass the BBB via β amino acid transporter TAUT, the so-called Taurine Transporter (Gebara *et al.,* 2015). Taurine is 3–4 times more represented in a developing brain than in an adult one, however, it declines gradually with aging (Banay-Schwartz *et al.,* 1989). Recently, taurine was documented to improve cognitive function and keep against neuropathology in an animal model of AD (Oh *et al.,* 2020). It was stated that taurine exhibited therapeutic potential against neurological disorders, including AD (Jakaria *et al.,* 2019; Oh *et al.,* 2020). It was documented that taurine connects to Aβ plaques with weak anti-fibrillogenic effects (Santa-Maria *et al.,* 2007). Furthermore, intravenously administered taurine avoids Aβ neurotoxicity and cognitive impairment (Su *et al.,* 2014). To date, no documents of the possible side effects of taurine have been reported, and due to its nontoxic characteristics in the body, it has been used as a nutrient in foods (Oh *et al.,* 2020). It also enhances memory and hippocampus-dependent learning and alleviates anxiety and depression (Si *et al.,* 2004). Taurine was found to enhance stem cell activation and propagation yielding a higher concentration of neural progenitors and

stem cells, and aid to lessen the number of activated microglia leading to down-regulated inflammation. (Gebara *et al.,* 2015). It was reported to enhance the propagation of adult neural stem/progenitor cells *in vitro* (Gerardo Ramos-Mandujano *et al.,* 2014). In view of the above-mentioned correlation of ADMSCs and taurine in treating AD, the present study aims to investigate the possible therapeutic effects of ADMSCs with/without 2-taurine on neurodegeneration in a male rat model of AD, in order to introduce an approved pre-clinical study that can be used in the future for advanced clinical studies.

MATERIALS AND METHODS

Fifty male Wistar rats (Rattus norvegicus), (weighing 120-150 g) (National Research Center, Dokki, Egypt) were allotted for the present study. Animals were acclimatized to the experimental conditions for 2 weeks before the onset of the experiment. Animals had free access to a standard pelleted diet specialized for rats with 20% protein (Agricultural-Industrial Integration Company, Giza, Egypt) and tap water. Rats were housed in plastic cages (five animals per cage and provided with bedding (sawdust) and kept in a room with standard illumination (12:12 h light: dark cycle) and at a constant temperature (25°C). Experimental protocols and procedures used in this study were approved by the Cairo University Institutional Animal Care and Use Committee (CU-IACUC) (Egypt), (approval no. CU/I/F/86/17) in accordance with the international guidelines for the care and use of laboratory animals.

1-Experimental Design:

The present study was planned to include three successive phases; induction phase (I), during which $AlCl_3$ dissolved in distilled water had been orally administered (100 mg/ kg BW/ per day) for consecutive 40 days, withdrawal phase (W) for 20 days during which $AlCl_3$ administration had been stopped, and therapeutic phase for 30 days (T) during which ADMSCs were injected once (10^6/ml DMEM) in tail vein, and/or taurine (150 mg/ kg BW/ per day) given by oral intubation for 14 days. After the acclimatization period, animals were randomly divided into 2 main groups: the control (C) group (15 rats) and Alzheimer's disease (AD) group (35 rats) (Table 1).

The control rats including the following subgroups: CI, CW & CT, were daily administrated distilled water by oral intubation during the induction and therapeutic phases.

Alzheimer's disease model (AD group) rats were orally administrated 100 mg/ kg/BW/ per day of $AlCl_3.5\ H_2O$ for 40 days (Induction phase). Thereafter, animals have passed a withdrawal phase for 45 days without any treatment (Withdrawal phase). At the end of the withdrawal period, the therapeutic phase started when 10 rats received a single dose of PKH67 green fluorescent-labelled ADMSCs (1.0×10^6 cells/ 0.5 ml serum-supplemented DMEM per rat) administered intravenously in the tail vein, while five of which rats have additionally received 150 mg/kg/day taurine for 35 days. In parallel, 5 rats from the control (C) group received 0.5 ml serum-supplemented DMEM per rat intravenously (CT). Five other rats from the AD group received 200 mg/kg/day of taurine only for 35 days. Accordingly, the current study has five groups in the therapeutic phase (ADT, ADTS, ADTT, ADTST, CT)

At the end of each experimental phase, rats of each subgroup were euthanized by cervical dislocation under anaesthesia (100 mg sodium pentobarbital). After dissection, blood and brain tissue samples were obtained; serum was separated and kept frozen at -20°C for subsequent assay. Half of the brain was fixed in 10% formalin for histopathological examination and homing of different brain areas, namely the cerebral cortex, cerebellum, hippocampus, and medulla oblongata. The other half was weighed,

homogenized in 1x phosphate buffer saline (PBS), and kept frozen at -80°C for molecular measurement.

Table 1: Experimental design including phases, groups, number of rats per group and different treatments.

Groups	No. of Rats (N)	Experimental phases
	N= 50	Acclimatization Period (7 days)
	N= 50	Induction Phase (30 days)
CI	N= 5	Daily administrated distilled water by oral intubation
ADI	N= 10 (-5*)	Orally administrated 100 mg/ kg/BW/ per day $AlCl_3.5\ H_2O$
	N= 35	Withdrawal Phase (45 days)
CW	N= 5	No treatment
ADW	N= 5	No treatment
	N= 25	Therapeutic phase (35 days)
CT	N= 5	Daily administrated distilled water by oral intubation
ADT	N= 5	No treatment
ADTS	N= 5	Single treatment with a single dose of ADMSCs at day (0) (1.0 x 106 cells/ 0.5 ml serum-supplemented DMEM per rat) administered intravenously in tail vein
ADTT	N= 5	Single treatment with 200 mg/kg/day taurine only for 35 days
ADTST	N= 5	Combined treatment with single dose of ADMSCs at day (0) (1.0 x 106 cells/ 0.5 ml serum-supplemented DMEM per rat) administered intravenously in tail vein + 200 mg/kg/day taurine only for 35 days
	∑= 50 rats (-5*) = dead rats	

2-Serum Sample Preparation:

Blood samples were collected in non-heparinized tubes and centrifuged at 3000 rpm (RCF = 1008 × g) for 20 minutes. Serum was stored at −20 °C for subsequent biochemical assays.

3-Brain Homogenate Preparation:

Half of the brain was freshly weighed and homogenized in 1x PBS. The supernatant was collected carefully after centrifuging for 20 min at 2,000-3,000 rpm and aliquoted for subsequent assays.

Behavioral Test:

1-T Maze Test:

Animals were exposed to a T-maze test to assess rats' behavior during the induction of AD, withdrawal, and therapeutic phases. The apparatus has three arms (20*30/60), and one of them had animal food at its end. Firstly, rats were encouraged to explore the routes and determine which one has the food at the end. At the beginning of the actual training, rats were placed at the center of the maze and left to explore to find the food while the time was being measured; the trial ends when rats reach the right arm to obtain the food reward. The main parameter was the time, as it takes rats to reach the food, the more deteriorated memory they have (Deacon et al., 2006).

2-Estimation of Acetylcholinesterase (AChE):

Serum AChE activity was assayed using the spectrophotometric kit of Biodiagnostic, Dokki, Giza, Egypt following the manufacturer's instructions. The absorbance change was measured using a kinetic method at 412 nm.

***3*-Measurement of the β-amyloid Beta Peptide by Enzyme-Linked Immunosorbent Assay (ELISA):**

According to the manufacturer's instructions, brain tissue and serum β-amyloid levels were measured by ELISA (ANOVA, Keyuan Road, Daxing Industry Zone, Bejing, China).

4-Oxidative Stress Markers Assessment:

Brain oxidative stress markers were detected using ready-made spectrophotometric kits of Biodiagnostic, Dokki, Giza, Egypt according to the manufacturer's instructions for lipid peroxidation (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), respectively.

5-Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR):

Total RNA was extracted from a frozen sample of brain homogenates of rat groups using an RNA Mini- preparation kit (Bio Basic Canada, Inc.). According to the manufacturer's instructions, the concentration and quality were tested using a Q-5000 ultraviolet spectrophotometer (Quawell, USA). According to the manufacturer's instructions, a total of 1 µg total RNA was converted into cDNA using the SensiFAST cDNA synthesis kit (Bio Basic Canada, Inc.). Then RT-qPCR was performed using the Maxima SYBR-Green Master Mix kit (Thermo Fisher Scientific, Inc.) to amplify p53, Bax and Bcl2 genes in parallel with the housekeeping gene glyceraldehyde 3- phosphate dehydrogenase (Gapdh). Primers used for qPCR were commercially synthesized by Macrogen, Inc. (Seoul, Korea) and are listed in Table 2. qPCR was performed in applied Biosystems Step One Plus (Thermo Fisher Scientific, Inc.), and reactions were done in triplicates. Each sample was initially denatured at 95 °C for 5 min and then subjected to 40 cycles of the following: denaturation at 95 °C for 50 s, and annealing and extension at 60 °C for 1 min. Each sample was exposed to a final extension at 72 °C for 10 min and finally was held at 4 °C followed by blotting the amplification and melting curves. The following qPCR, Ct values, ΔCt, ΔΔCt, and fold expression was calculated to quantify the results (Livak and Schmittgen, 2001).

6-Homing of ADMSCS in Brain Tissues:

One rat from the ADTS group was injected intravenously with a single dose of labelled 1 x 106 ADMSCs via a tail vein. *In Vivo*, Fluorescence measurements were done two days post-injection, and fluorescence images were observed using (an Olympus microscope) for data analysis.

7-Histopathological Examination:

Brain tissue samples were excised immediately after dissection, washed in ice-cold saline, dried on tissue paper, and fixed in neutral buffered formalin (10%) for further processing by the ordinary routine work: dehydration, clearing, and embedding. The brain tissues' paraffin-embedded blocks were cut using microtome at 6 µm- thick tissue sections and then stained with Haematoxylin and eosin (H&E) (Cardiff et al., 2014).

Statistical Analysis:

Values were expressed as (means ± SE). Statistical analysis was performed using SPSS statistical software package version 20 by one-way analysis of variance (ANOVA) with Duncan post hoc test, and $P \leq 0.05$ was considered statistically significant.

RESULTS

1-Bodyweight, Brain Weight, And Somatic Brain Index:

As recorded in Figure 1, ADI, ADW, and ADT exhibited a significant

decrease in body weight and brain weight compared to CI, CW, and CT during induction, withdrawal, and therapeutic phases, *indicating the continuous effect of Al intoxication*. During the therapeutic phase, ADTT and ADTST revealed a significant increase in body weight and brain weight compared to ADT, but an insignificant increase in ADTS compared to ADT. *It is worth mentioning here that ADTST showed the best improvement in rats' body weight compared to both ADTS and ADTT*.
Besides somatic brain index, ADI and ADW showed an insignificant decrease as compared to CI and CW during both the induction and withdrawal phases. During the therapeutic phase, ADT showed a significant decrease as compared to CT. In opposition, ADTS, ADTT and ADTST exhibited an insignificant increase in brain somatic index in comparison with ADT.

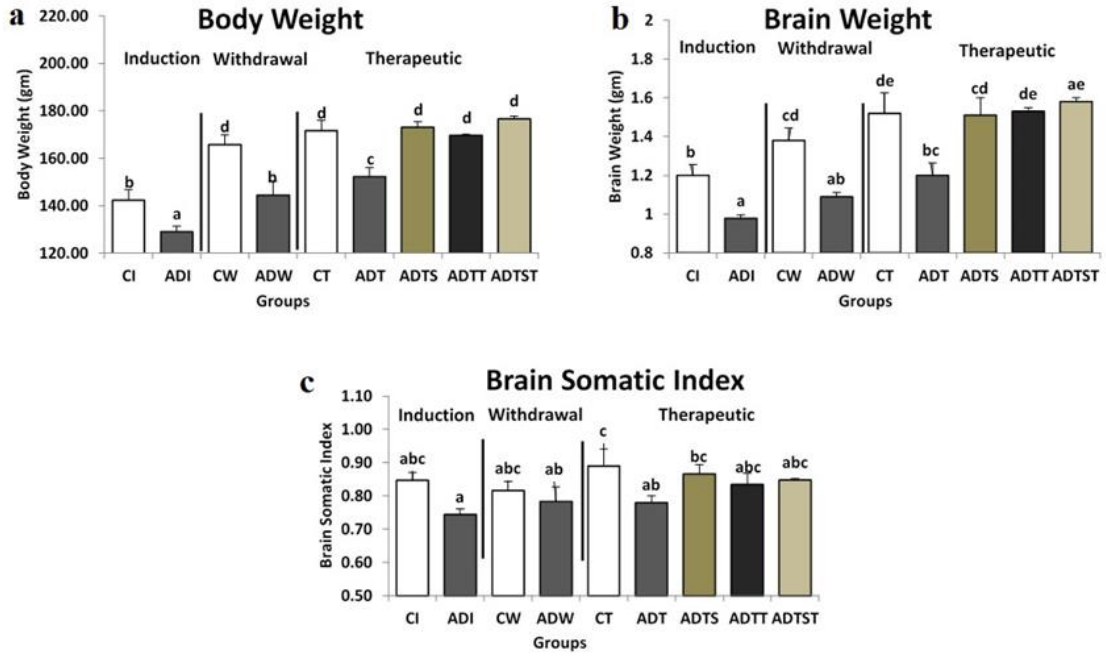

Fig. 1: Variations in (a) body weight, (b) brain weight, and (c) brain somatic index in AD rat model throughout the whole experimental period, the expression of values is using mean ± SE; P≤0.05 significant difference compared to both control and treated groups.

2-Behavioral Test:

2.1.T-Maze Test:

As illustrated in Figure 2, ADI, ADW, and ADT showed a significant increase in the time taken to reach the correct arm respective to the control rats (CI, CW, and CT) in both trials during induction, withdrawal, and therapeutic phases. *These findings might indicate the continuous effect of Al neural intoxication at the behavioral level.* During the therapeutic phase, all treated groups (ADTS, ADTT, and ADTST) showed a significant reduction in the time taken to reach the correct arm compared to ADT in both trials. Hence, all therapeutic groups demonstrated gradual improvement in rats' performance in both trials, but ADTST has shown the best effect, nearly reaching the control value in the second trial.

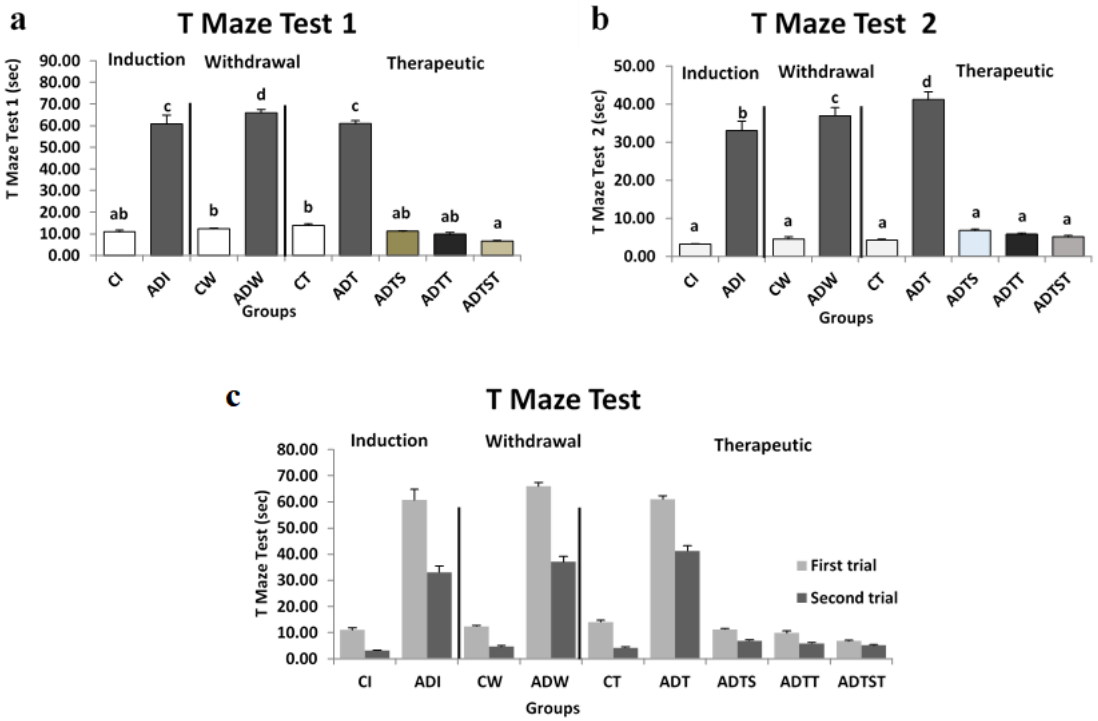

Fig. 2: Variations in the behavior of rats as manifested by the T-Maze test, describing the (a) first trial, (b) second trial, and (c) both trials in AD rat model throughout the whole experimental period, the expression of values is using mean ± SE; P≤0.05 significant difference compared to both control and treated groups.

3-Acetylcholinesterase and β-Amyloid Measurements:

As demonstrated in Figure 3a, ADI, ADW, and ADT showed a significant increase in serum AChE respective to control groups (CI, CW, and CT) during induction, withdrawal, and therapeutic phases. *These findings might indicate alterations in the transmission of nerve impulses in the brain and signs of depression.* During the therapeutic phase, ADTS, ADTT, and ADTST showed a significant reduction in serum AChE, nearly reaching the control values.

As observed in Figure 3b, ADI, ADW, and ADT have demonstrated a significant increase in brain and serum β- amyloid compared to CI, CW, and CT during all three experimental phases. *These data might indicate the progressive nature of Alzheimer's disease and signs of dementia by β-amyloid plaques accumulation even after withdrawal of AlCl3.* During the therapeutic phase, ADTS, ADTT, and ADTST showed a significant decrease in serum β- amyloid compared with ADT, but an insignificant decrease in brain β- amyloid of ADTT versus ADT. It is worth mentioning here that ADTST showed the best results in brain values among the three treated groups, while ADTS demonstrated the best results in the serum values that were nearly compatible to control values.

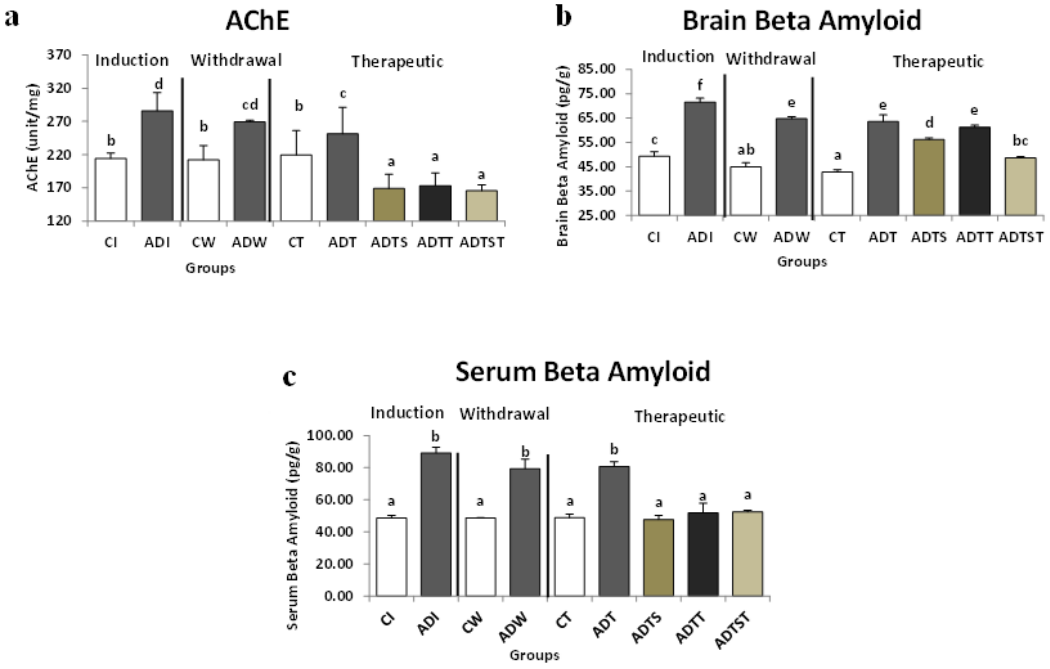

Fig. 3: Variations in a) serum ACHE concentrations, b) brain beta-amyloid concentrations, and (c) serum beta-amyloid concentrations in AD rat model throughout the whole experimental period, the expression of values is using mean ± SE; P≤0.05 significant difference compared to both control and treated groups.

4-Oxidative Stress Bioindicator:

Regarding oxidative stress markers in the brain, Figure 4 showed a significant increase of MDA in ADI, ADW, and ADT compared to CI, CW, and CT during all experimental phases. During the therapeutic phase, ADTS, ADTT, and ADTST showed a significant decrease in MDA versus ADT. Concerning anti-oxidative molecules, ADI and ADW showed a significant increase in GSH, and CAT compared to CI and CW, but an insignificant increase of SOD than CI and CW during both induction and withdrawal phases. During the therapeutic phase, ADT showed a significant increase in GSH, CAT, and SOD than CT. On the other hand, ADTS and ADTST showed a significant decline in GSH and CAT levels versus ADT, but an insignificant reduction of SOD in ADTT compared with ADT.

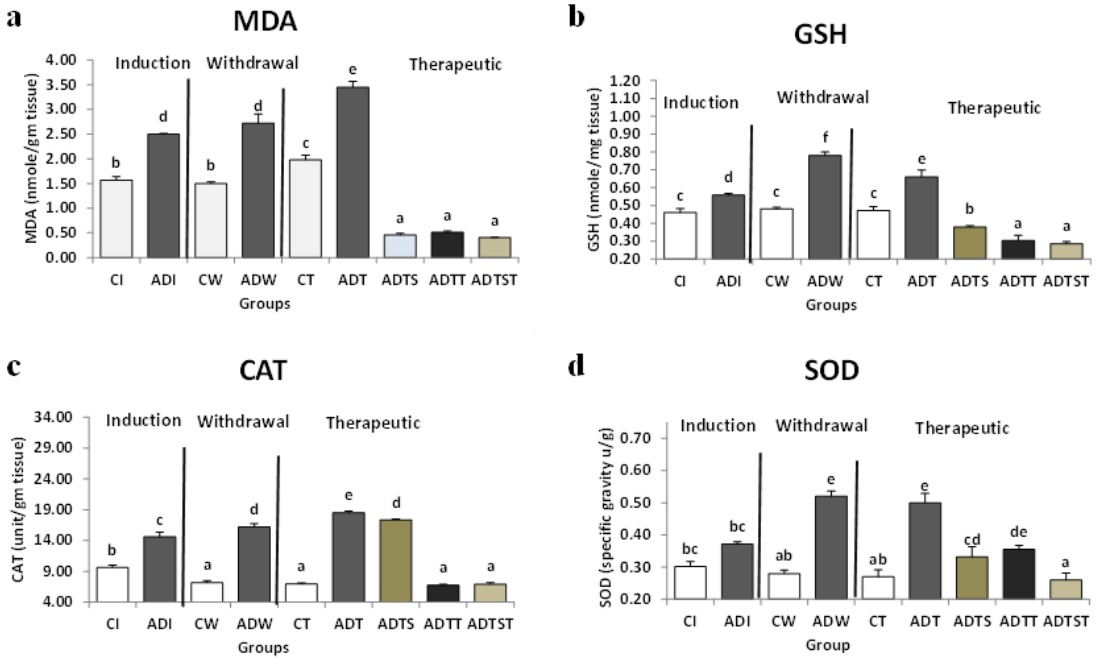

Fig. 4: Variations in brain (a) MDA (b) GSH (c) SOD and (d) CAT in AD rat model throughout the whole experimental period, the expression of values is using mean ± SE; P≤0.05 significant difference compared to both control and treated groups

5-Expressions of Pro-Apoptotic and Anti-Apoptotic Genes in Brain Tissues*:*

As demonstrated in Figure 5, ADI, ADW, and ADT showed a significant increase in p53 and Bax genes' expression levels compared to CI, CW, and CT during all experimental phases, *which may be one of the signs of Al neural intoxication.* ADTS, ADTT, and ADTST showed a significant decrease in pro-apoptotic markers' expression levels during the therapeutic phase compared with ADT. Regarding the anti-apoptotic marker (Bcl2), ADI, ADW, and ADT demonstrated a significant decrease in their expression level compared to CI, CW, and CT during the three experimental phases. ADTS, ADTT, and ADTST showed a significant increase in their expression level during the therapeutic phase compared to ADT. Although the three therapeutic groups enhanced p53 and Bax downregulation and Bcl2 upregulation versus ADT, it was apparent that ADTST exerted the best therapeutic effect with the expressions nearly reaching the respective control values.

6-Homing of ADMSCs in Brain:

The present study has successfully shown the unique characteristic of ADMSCs being homed to the site of injury (brain tissue), referring to their potential to cross the BBB as illustrated in **Figure 6.**

7-Histopathology:

As seen in Figure 7 and Figure 8, the present study illustrated the signs of neurotoxicity in the form of neuronal degeneration, focal amyloid plaques, and gliosis with haemorrhage around cerebral blood vessels in the cerebral cortex in ADI rats in comparison with control ones. ADTS, ADTT, and ADTST have shown partial enhancement in brain tissues' neural regeneration versus control rats.

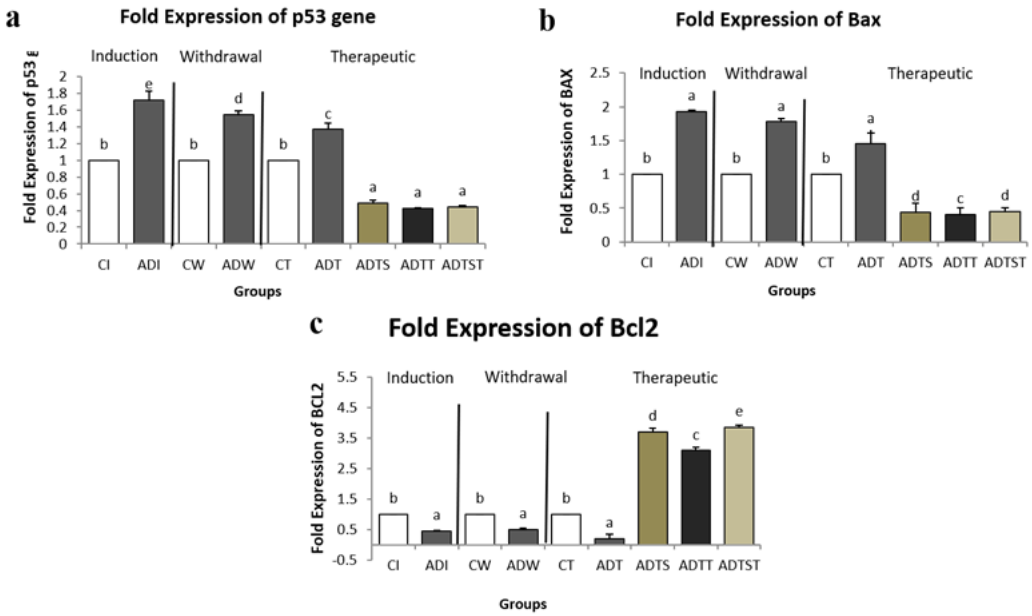

Fig. 5: Fold expressions of brain pro-apoptotic markers (a) p53 (b) Bax, and anti-apoptotic marker (c) Bcl2 in AD rat model throughout the whole experimental period, the expression of values is using mean ± SE; P≤0.05 significant difference compared to both control and treated groups.

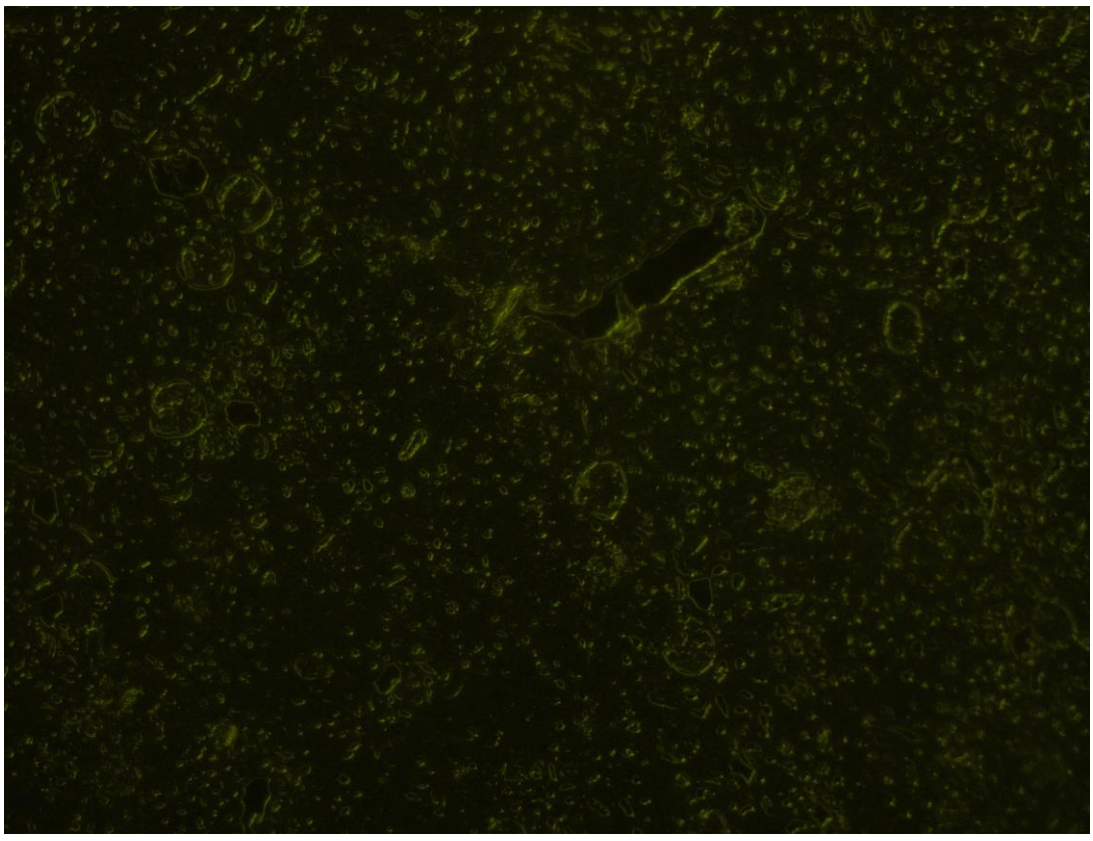

Fig. 6: Homing of fluorescent ADMSCs in the brain of AD rat model 2 days post-ADMSCs injection.

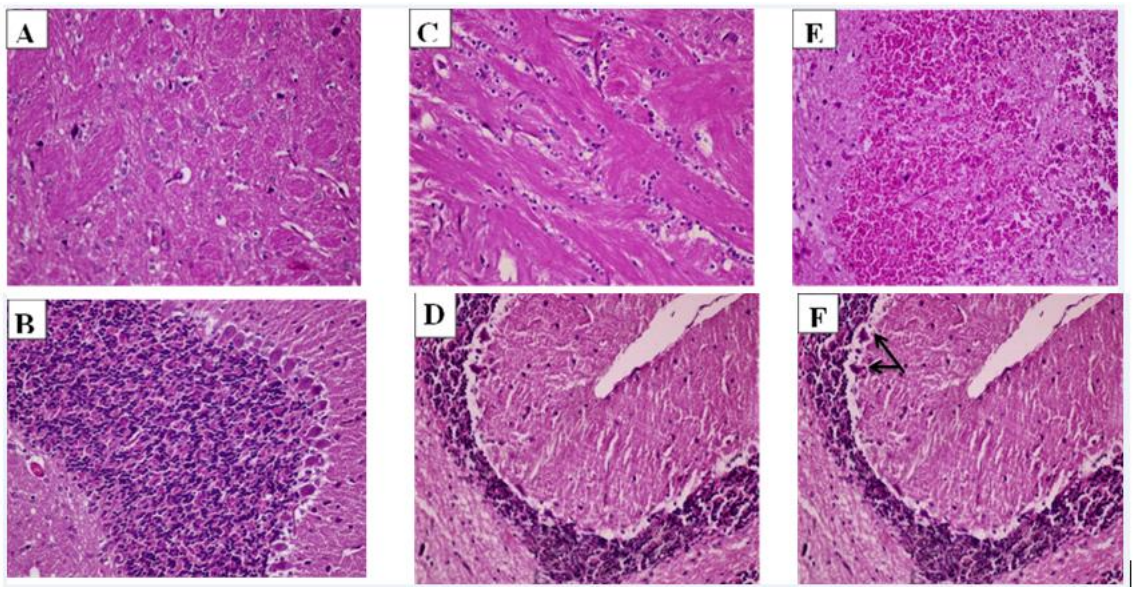

Fig. 7: Sections were taken from the brains of control rats at the end of the induction phase showing normal histological structures stained by Haematoxylin. Eosin stain of (A) cerebrum striatum (X400), and (B) cerebellum cortex (X400) respectively, and sections taken from the brains of rats given aluminum chloride at a dose of (100 mg/kg) at the end of the induction Phase showing (C) Cerebral Striatum showing focal gliosis (H&E X400) (D) Cerebellum showing necrosis of most of the Purkinje cells (arrow) (H&E X400), and sections taken from the brains of rats during AlCl3 withdrawal phase for 20 days (ADW group) are showing: (E) cerebral cortex with severe patches of haemorrhage and gliosis respectively (H&E 400X), and (F) cerebellum with absence of most of the Purkinje cells (Arrows, H&E 400X).

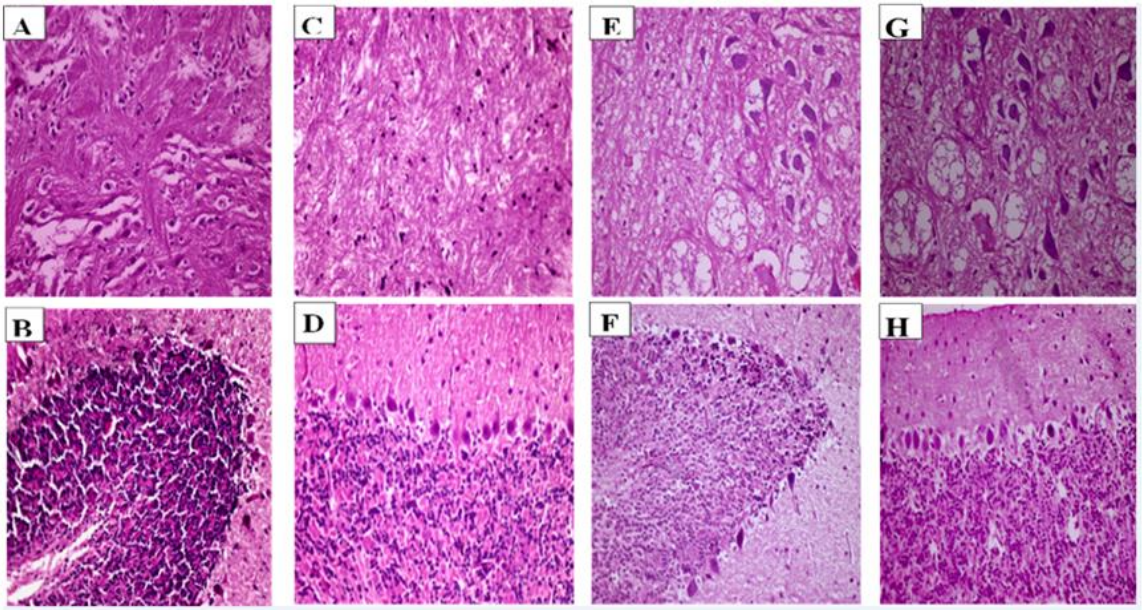

Fig. 8: Sections taken from the brains of rats after additional 35 days of AlCl3 withdrawal (ADT group) are showing: (A) cerebral striatum with neuronal degeneration and gliosis (H&E 400X), and (B) cerebellum with severe degeneration of the Purkinje cells (H&E 400X), sections were taken from the brains of rats treated with ADSCs intravenously as a single dose of (1 x 106 cells) after 35 days showing: (C) Cerebral Striatum showing mild encephalomalacia and gliosis (H&E X400). (D) Cerebellum showing typical appearance of Purkinje cells (H&E X400), sections were taken from the brains of rats treated with Taurine at a dose of (150 mg taurine/kg BW/ per day after 14 days showing: (E) Medulla Oblongata showing encephalomalacia (H&E X400). (F) Cerebellum showing degeneration of most of the Purkinje cells (H&E X400), sections were taken from the brains of rats treated with Taurine at a dose of (150 mg taurine/kg BW/ per day + ADSC at a dose of (1 x 106 cells) after 60 days showing: (G) Medulla Oblongata showing encephalomalacia (H&E X400). (H) Cerebellum showing mild degeneration of Purkinje cells (H&E X400).

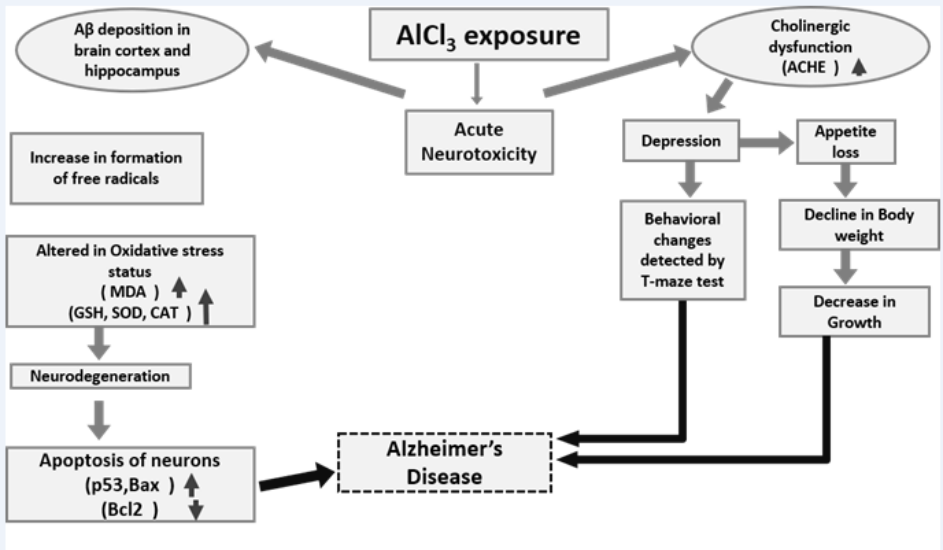

Fig. 9: Schematic representation of Alzheimer's disease signs after $_{AlCl3}$ induction in the rat model.

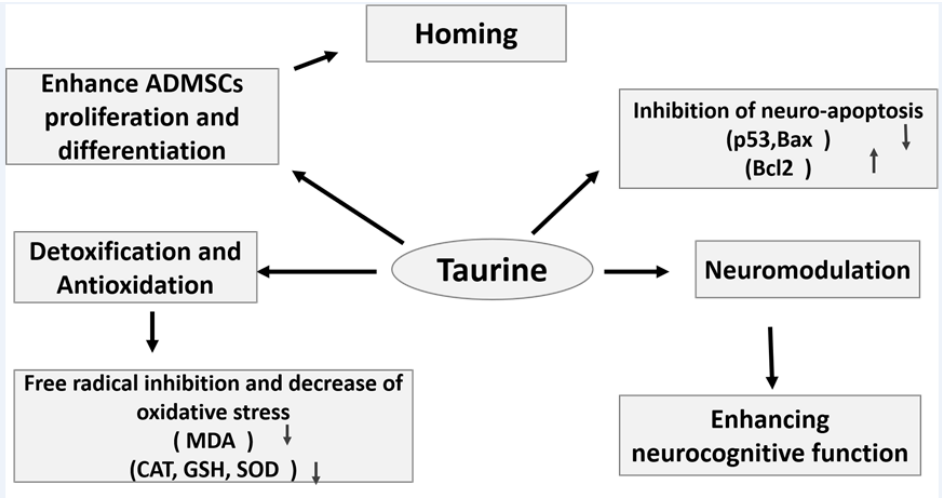

Fig. 10: Schematic representation of the compensatory effect of an administration of Taurine in rat model.

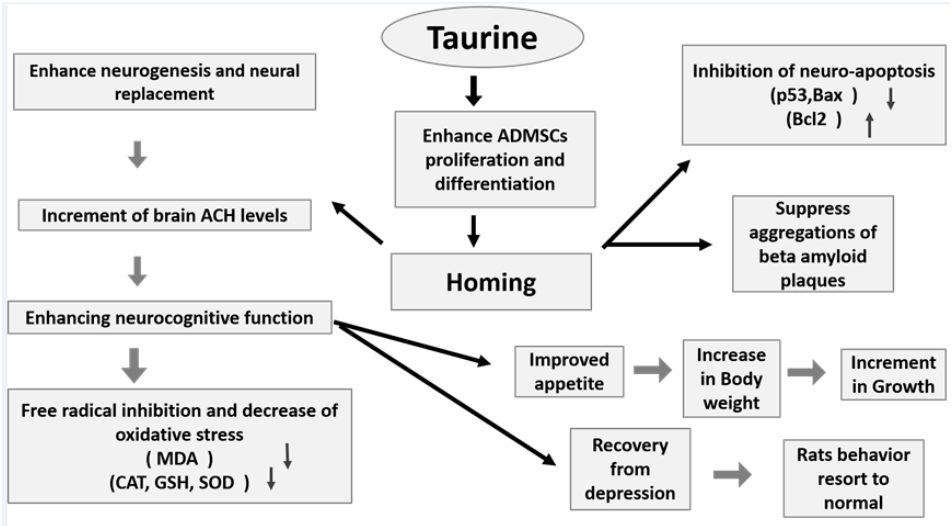

Fig. 11: Schematic representation of the compensatory effect of an administration of ADMSCs and/or Taurine in rat model.

DISCUSSION

The exploration for effective AD treatment is a challenge for modern nanomedicine and neurobiology due to the increase of several disturbances at the cellular level and variations in the physiology of the action (Xiao *et al.,* 2017). The recently used traditional treatments for AD have limited medicinal abilities, so finding a new therapy is a critical challenge that faces scientific research. However, these traditional treatments can enhance symptoms; they cannot stop AD progression, and they only make symptomatic relief with some side effects (Xiao *et al.,* 2017).

Stem cell therapy in regenerative medicine is one of the promising approaches to curing neurodegenerative diseases, especially AD (Kim *et al.,* 2012). Adipose-derived mesenchymal stem cells (ADMSCs) are documented for their effective preventive and therapeutic potentials in stem cell-based therapy and regenerative medicine. According to Kim *et al.* (2012), MSCs exhibit the ability to cross the blood-brain barrier after the intravenous injection and efficiently migrate to neural injury areas without induction of tumorigenicity or immune response (Ra *et al.,* 2011). AMSCs also have an immune regulatory paracrine effect, including overexpression of neuroprotective cytokines like IL-10 and decreased levels of pro-inflammatory cytokines IL-1β and TNF-α (Yang *et al.,* 2013; Kim *et al.,* 2013), therefore, they can ameliorate the progression of the AD (Kim *et al.,* 2012).

On the other hand, Taurine is reported to enhance the proliferation of stem cells in the dentate gyrus by increasing the number of intermediate neural progenitors (Gebara *et al.,* 2015). Taurine reduced the number of activated microglia, which means a lower level of inflammation and prolonged survival for the new neuron (Zheng *et al.,* 2017). The present study aimed to investigate the therapeutic potential of ADMSCs and/or Taurine against AlCl3-induced Alzheimer's disease in the rat model.

In the present study, several behavioral and physiological impairments have been documented in the AlCl3 model of Alzheimer's disease, which has been reported to be partially ameliorated by the administration of ADMSCs and /or taurine administration. The current study successfully induced AD in the rat model by oral intubation of AlCl3 in the dose used, which was evident from the signs of dementia, T-maze behavioral test accompanied by loss of appetite, and decline in body weight. These signs were also documented at the physiological level as demonstrated by enhancement of serum AChE activity, accumulation of β- amyloid in the brain and serum, increment in oxidative stress marker (MDA), and decline in the antioxidant defense system in the brain. At the molecular level, there was an increment in apoptotic markers, confirmed by signs of degeneration in various brain areas at the histopathological level.

There was an overall reduction in body weight and brain weight in ADI and ADW groups versus the respective control value even after AlCl3 has been withdrawn in the present study. *The current results might support additional evidence that Alzheimer is a progressive disease* (Lane *et al.,* 2017; De Ture et al., 2019). During the therapeutic phase, body weight, brain weight, and somatic brain index were partially restored after administration with ADMSCs and/or Taurine compared to the control group. These findings were parallel to the study documented by Thenmozhi *et al.* (2015) in ADMSCs treated AD rat model.

The current study has also demonstrated the signs of neurodegeneration in the AlCl3-induced AD rat model in terms of locomotion, anxiety, activity, and behavior, as documented by (Abdel-Wahab 2012). These signs appear to be associated with the accumulation of AlCl3 in the rat brain, as supported by (Thenmozhi *et al.,* 2015). Behavioral test as T-maze test showed improvement in learning and recognition in the

treated groups compared to the AlCl3 model group and agreed with previous results (Zheng *et al.,* 2017).

Acetylcholine esterase plays a critical role in the process of acetylcholine hydrolysis, which is a cholinergic neurotransmitter that has a deep relation to learning and memory. So higher acetylcholine esterase and resultant accelerated hydrolysis produced fewer acetylcholine neurotransmitters directly proportional to AD progression (Zheng *et al.,* 2017). The present study demonstrated that AchE levels were higher in AD group than control during both the induction and withdrawal phases. During the therapeutic phase, AchE has shown remarkable inhibition in the three treated groups due to ADMSCs and /or taurine administration. These current findings ran in parallel in the case of ADMSCs with those documented by (Zheng *et al.,* 2017) and supported their potential role in re-establishing motor activity in the rain.

One of the leading causes of AD is the accumulation of the β-amyloid plaques in the brain with consequent alteration of the signaling process, leading to brain dysfunction. The current study reported that β-amyloid concentration levels were increased in the AD group compared to the control one during the induction and withdrawal phases, and that agrees with previous results (Kim et al., 2012; Gebara et al., 2015) during the induction phase. These current findings have also been supported by the histopathological findings in the current study, illustrating the accumulation of focal amyloid plaques in the cerebral cortex and hippocampus. Once again, this supports that Alzheimer is a progressive disease. Under the current experimental condition, administration of ADMSCs has succeeded in significantly reducing the accumulation of β-amyloid plaques during the therapeutic phase against ADT group. These current findings ran in parallel with those previously documented by (Kim *et al.,* 2012), who proved the capability of multiple administration of ADMSCs to dramatically decrease serum β-amyloid plaques after four months in the AD-mice model.

Oxidative stress is an imbalance between oxidants and antioxidants choosing the oxidants and plays a vital role in AD pathogenesis. Mitochondrial dysfunction and oxidative stress are strongly associated with AD and other neurodegenerative diseases (Guo *et al.,* 2013). Oxidative stress plays a vital role in Aβ breakdown and clearance (Cheignon *et al.,* 2017). Furthermore, oxidative stress causes neuropathological changes, namely neuronal apoptosis and the formation of neurofibrillary tangles and β-amyloid aggregation, which eventually leads to AD progression (Bokov *et al.,* 2004). In the current study, it was found that brain levels of MDA, CAT, GSH, and SOD activities were significantly higher in the AD model group than in the control group during both the induction and withdrawal phases. By proceeding to the therapeutic phase, it was found that MDA was reduced in the three treated groups compared to the control. GSH and CAT showed a significant decrease in ADTS and ADTST, but SOD showed an insignificant decline in ADTT compared to control. The current results ran in parallel with previous studies done by (Bokov *et al.,* 2004), which might reflect a potential role of ADMSCs in restoring oxidative balance in the AD rat model.

AD is a neurodegenerative disease that looks to be due to prompting apoptosis (Obulesu *et al.,* 2014). Hence, the current study evaluated the mRNA expression of pro-apoptotic markers (p53 and Bax), and anti-apoptotic marker (Bcl2) genes to confirm this hypothesis in terms of quantitative gene expression. The present study showed a significant increase in p53 and Bax genes' expression level and downregulation of Bcl2 in ADI and ADW compared to CI and CW during both the induction and withdrawal phases. These current findings are documented by the previous reports of Liu *et al.* (2010) during the induction phase, which seems to be due to Al intoxication. During the therapeutic phase, the condition has been reversed where p53 and Bax have been

downregulated, while Bcl2 has been upregulated significantly in the three treated groups as compared to the control. These current findings might reflect the potential role of ADMSCs in enhancing neuro-regeneration in AD rat models through the remarkable inhibition of apoptosis. Regarding homing, the present study has also confirmed the claims of (Kim *et al.*, 2012) that ADMSCs can cross the blood-brain barrier and home at the injury site (brain).

In conclusion:

As illustrated in figures 9, 10 and 11, the current study successfully induced AD in the rat model by oral intubation of AlCl3 in the dose used, which was evident from the signs of dementia, T-maze behavioral test attended with loss of appetite and decline in body weight. These signs were also proved at the physiological level as demonstrated by an increment in AChE activity, accumulation of β- amyloid, increment in oxidative stress marker (MDA), and decline in the antioxidant defense system. At the molecular level, there was an increment in apoptotic markers manifested by signs of degeneration in various brain areas at the histopathological level. The current study has also documented that injection of a single dose of ADMSCs enhanced the appetite of AD rats, which enhanced their body weights, compensated the behavioral disorders correlated with Al neurotoxicity, and re-established the motor activity in the brain. Furthermore, administration of ADMSCs reduced β-amyloid plaques at the therapeutic phase and counteracted the harmful effects of Al toxicity on the oxidative balance in brain tissues. Furthermore, the intervention with ADMSCs led to a significant enhancement of neuro-regeneration in AD rats via the inhibition of apoptosis. Ultimately, the current data has demonstrated the therapeutic potential of a single administration of ADMSCs for partial compensation of various AD symptoms in a rat model at behavioral, physiological, molecular, and histopathological levels under experimental conditions.

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Consent to participate: (not applicable)

Consent for publication: (not applicable)

Availability of data: The data used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability: (not applicable)

Authors' information: Mohamed Hosney and Alaa Sakraan contributed equally to this work**.**

Authors' contributions: Emad M Elzayat and Mohamed Hosney have conceived, designed, planned, and supervised the research point. Emad M Elzayat, Mohamed Hosney and Alaa Sakraan performed the experimental work. Emad M Elzayat and Mohamed Hosney analyzed and interpreted the data. Mohamed Hosney, Alaa Sakraan, and Emad M Elzayat participated in writing the manuscript. Mohamed Hosney and Emad M Elzayat participated in summarizing the findings in a schematic diagram, and reviewed, and edited the final version of the manuscript. All authors approved the final version of the manuscript.

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