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The Neuro Engraftment and Neuroregenerative effects of Hydrogen Sulphide Donor, Intracerebral MSCs, Ginko Biloba and Kefir in Attenuating Neuropathological hallmarks of Lipopolysaccharide induced Alzheimer's disease Rat models

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ABSTRACT

Background: Memory disorders have been characterized by being a devastating long term incurable diseases with a huge social impact in addition to a diminished efficient available medical treatments. Deep Brain stimulation via using neuroprotective inducers for treatment of brain structure degenerative diseases such as Alzheimer's disease (AD) can be considered as being a promising successful therapy due to its various targets and underlying mechanisms for improving brain dysfunction. **Objectives:** The main aim of this study is to suggest therapeutic protocol having the potentials for restoring normal neurons diverse population and modifying neuropathological deposited hallmarks including both positive and negative lesions. **Materials and Methods:** Rats were divided into nine groups: (G1) control ;(G2) rats received LPS as a method of inducing nongenetically manipulated AD;(G3)AD rats received NaHS;(G4) AD rats received MSCs intracerebrally;(G5) AD rats received MSCs+NaHS;(G6)AD rats received kefir+GB;(G7)AD rats received MSCs+kefir+GB;(G8)AD rats received NaHS+kefir+GB; (G9) AD rats received MSCs+NaHS+kefir+GB. **Results:** AD induction resulted in down-regulation of CBS expression and GSH brain tissue level accompanied with overexpression in amyloid- β protein, MAPK, tau protein, ACAT expression and MDA brain tissue level in addition to elevated caspase-3 serum level. **Conclusion:** The implantation of amyloid reliving therapy that do have a wide clinical impact if initiated at benign plaques stage before irreversible brain damage occurs. The following effects have been observed following the administration of suggested medical protocol where a decrease in AD pathological deposited hallmarks has been observed with maintaining inflammatory brain factors by functioning as a potent neuroregenerative.

Keywords: LPS induced rats, MSCs, hydrogen sulphide, Kefir, amyloid β , intracerebral.

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Introduction

Neuroregeneration is a continual regrowth/repairing processes for various types of nervous cells and damaged brain tissues whether located in the peripheral or central nervous system for restoring normal brain function abilities and maintained synaptic transmission system. A clear understanding of a neuro-regenerative functions and the relation that can be established for restoring the attenuated mental abilities in neurodegenerative diseases such as Alzheimer's disease (AD) must be clearly stated to clarify its efficiency. Alzheimer's disease is the most common type of dementia characterised by altered behavioural symptoms, memory loss, aphasia, cognitive deterioration and apraxia [1]. Difficulty with accepting new information and memory recall failure are the most common drawbacks of numerous amyloid- β aggregations and neurofibrillary tangles located intracellularly within the brain of AD patients [2-3]. Foreign substance identified as fibrillated amyloid- β peptides can oligomerize and aggregate forming diffuse plaques (benign plaques) which on the long run turn into neuritic plaques (irreversible form) being produced by processing amyloid precursor protein (APP) via β - and γ -secretases enzymes respectively leading to synaptic dysfunction and neuronal cell death. The processing of APP to A β amyloidogenic pathway can be enhanced by overlapping inflammatory signals pathways and mediators including apoptotic regulator factors, oxidative stress and excessive lipid foam cells [4-7]. Alzheimer's disease can also be related to an observed decreased in frontal and temporal lobe metabolism characterized by atrophy of cortex and hippocampus brain regions with defective alterations in memory networks and neuronal circuitry [4-5]. Thus, AD can be newly defined as a degenerative mental disease with over all systemic immunological body disorders affecting various biological integrated pathways leading to exaggerated unmaintained proinflammatory triggering factors [6-7].

Lipopolysaccharide as an inducer of nongenetically manipulated AD type

Toll-like receptors (TLRs) are evolutionary conserved type I integral membrane glycoproteins which can sense either pattern associated molecular patterns (PAMPs) and/or damage-associated molecular patterns (DAMPs). Lipopolysaccharides (LPS) is the major inducer of pro-apoptotic factors, inflammatory cytokines, chemokines, prostaglandins, and nitric oxide. LPS/TLR4 induce endotoxin cascade starts with recognition of PAMPs by pattern recognition receptor (PRR), activating several specific targeting proteins including cluster of differentiation 14 (CD14), MD2 and Toll/IL-1 receptor(TIR) domain [8]. The activation of the TLR4/MD-2 complex after binding LPS leads to the initiation of proinflammatory signalling components evoked by intracellular neuronal activation of tumor necrosis factor-alpha (TNF- α), nuclear factor kappa B (NF- κ B), mitogen activated protein kinase (MAPK) and c-Jun N-terminal kinases (JNK activation [8-9].

Exogenous hydrogen sulphide donor and AD

The out breaking discovery of endogenously produced H₂S in the brain prompted us to find out the enzyme responsible for its production which found out to be mainly produced by cystathionine β -synthase (CBS) pyridoxal-5-phosphate-dependent enzyme predominantly found in the central nervous system [10]. Hydrogen sulphide (H₂S) is a neuromodulator cytotoxic gas which has the ability to protect neuron cells and synapse from apoptotic regulators, inflammatory cytokines and oxidative stress. The production of H₂S in the brain is mainly enhanced in response to regular neuronal excitation via the Ca²⁺ and calmodulin-mediated pathways potentiating neurotransmission by acting as a neuromodulator influencing N-methyl-D-aspartate (NMDA) receptors and corticotropin-releasing hormone from the hypothalamus [10]. Potential therapeutic value of H₂S was clearly found to be useful in several

neurodegenerative diseases including AD, Parkinson's disease and traumatic brain injury via acting as neuroprotectant, antioxidant, anti-inflammatory, and antiapoptotic [11]. Based on these observations, H₂S production is suppressed by AD accompanied aggregations as a result of a proportional inhibition of CBS enzyme. Meanwhile our resent study provides a suggestion for alternative exogenous source of hydrogen sulphide to compensate its deficiency following AD hallmarks disposition in addition to preventing AD predicted progression and undesirable drawbacks.

Intracerebral MSCs and AD

Mesenchymal stem cells (MSCs) can be defined as a promising adjuvant cell regenerative therapy which can be used in various diseases including AD whether administrated I.V systemically or localized intracerebrally via tissue repair mechanism and regenerating tropic factors releasing strategy such as brain-derived neurotrophic factor (BDNF) based upon the origin and innate characteristic functions of the stem cells [12-14]. Neurons cells repair, decreased apoptotic inflammatory factors, reduced free radicals level and activated synaptic connection from damaged neurons were observed mainly upon MSCs brain transplantation by the ability to engulf A β aggregates which can be mainly attributed to microglial cell activation [13,14].

Ginko Biloba and AD

Natural products have been recently investigated and used as a regenerative therapy for their ability to induce cells neural proliferation, differentiation and brain function integrity. A combination therapy of kefir and *Ginko Biloba* (GB) have been used in the present study targeting their beneficial complex pathology for the treatment of AD mental disorders due various therapeutic mechanism of action [15-17]. *Ginko Biloba* (GB) can promote neurogenesis in brain trauma and injury conditions by augmenting physiological neural repair mechanisms through the release of growth and tropic factors [18-20]. Most of the

neuroprotective actions of GB are so-called polyvalent actions including antioxidant and anti-inflammatory activity via increasing cerebral blood flow and modulating neurotransmitter activity which can be linked to flavone glycosides and terpenoids active constituents [21]. *Ginko Biloba* exerts also a beneficial effect against amyloid beta (A β)-induced free radical-mediated neurotoxicity hypothesis through the stimulation of NGFR (nerve growth factor receptor)/PI3 kinase-mediated cell survival pathway, inhibition of MAPK cascade, antioxidant free radical scavenger activity and anti-apoptotic actions [18-21].

Milk Kefir grains and AD

Kefir is a cultured fermented milk product produced using milk kefir grains, where it found to produce a slightly acidic and foamy milky drink. Kefir grains consist mainly of lactic acid and acetic acid bacteria (lactobacilli, lactococci, Leuconostoc) and yeasts, which coexist as a complex symbiotic association in a protein-polysaccharide matrix [22]. Kefir probiotic microorganisms can exert their several health-beneficial functions as neuromodulator, immunostimulant, anti-inflammatory and antioxidant effects through wide different mechanisms including complete competitive exclusion or inhibition of pathogenesis, immune response modulation and regulation of metabolic functions [23-25].

The main aim of the current study is to provide a safe quaternary combination therapy composed of mesenchymal stem cell and/or NaHS and/or kefir and GB with the ability to restore mental and behaviour abnormalities as a results of AD hallmarks brain disposition within neural cells by neural ravishing regeneration mechanisms, restoring synaptic transmission activity , releasing tropic factors and attenuating pro-inflammatory triggering agitators with the advantage of being easily administrated and implemented on human research subjects to be used clinically.

2. Materials and methods

2.1. Materials used

Sodium hydrogen sulphide (NaHS) and Lipopolysaccharides (LPS) were purchased from (Sigma-Aldrich, St. Louis, MO), GB extract was obtained from Pharaonia Pharmaceuticals Egypt, dried Kefir grains were purchased from Cultures for Health (17978 S, Grasl Rd. Ore. City OR 97045, United States), Dulbecco's Modified Eagle's Medium (DMEM) and Phosphate buffered saline (PBS) were purchased from (Lonza) and RNeasy lysis buffer (RLT) (QIAGEN).

2.2. Preparation of milk Kefir grains:

20 mg of dried kefir grains were inoculated in 100 ml of pasteurized milk following by incubation for 24 h at 20°C. At the end of fermentation, milk was filtered to remove kefir grains [12,26].

2.3. Preparation of *Ginko Biloba* (GB):

Rats administrated 100mg/kg single daily dose of GB by oral gavage for 15 days with slight dose modifications [27].

2.4. Preparation of exogenous hydrogen sulphide (NaHS):

Rats received NaHS intraperitoneally (IP) at a daily dose of 5 mg/kg dissolved in deionized water for 15 days with slight dose modification [28].

2.5. Preparation of Bone Marrow (BM) derived MSCs:

Mesenchymal stem cell were isolated by flushing tibia and femur with DMEM from five adult male albino rats in accordance with density gradient [Ficoll/Paque (Pharmacia)] followed by re-suspension with 1% penicillin-streptomycin and 10% fetal bovine and left to be incubated at 37°C for 12–14 days in 5% humidified CO₂ till the formation of large colonies followed by direct twice times washing with PBS (pH7). Cells were then trypsinized with 0.25% trypsin in 1mM EDTA at 37°C for 5 min then incubated in 50 cm² culture flasks following centrifugation. MSCs were identified by adhesiveness, fusiform characteristic shape and ability to differentiate into osteocytes and chondrocytes (Jaiswal *et al.*,

1997). The morphological characters of cell surface molecules expression were analyzed using flow cytometry procedures for CD45-ve, CD 90, and CD105+ve as shown in figure 1(A).

2.6. Identification and labeling of BM-MSCs

Bone marrow derived mesenchymal stem cells were labeled with PKH26 red fluorescence cell linker kit prior to being transplanted according to the manufacturer's recommendations (Sigma, Saint Louis, Missouri, USA). Cells were centrifuged, washed, pelleted and suspended respectively in PKH26 dye solution. One month post transplantation, labeled MSCs with PKH26 were tracked and visualized using a fluorescence microscope (Sigma-Aldrich, Saint Louis, USA) as shown in figure 1(B).

2.7. Animals

Seventy-two (72) male albino rats, weighing 200- 250 g were purchased from the experimental animal facility unit, Faculty of Medicine, Cairo University. Rats were maintained in sterile pathogen free controlled temperature animal house at 22°C in a 12-h light/dark cycle with free access to water and semi-purified diet containing (g/kg): 100 g casein, 750 g sucrose, 50 g cellulose, 50 g fat blends, 10 g vitamin mix, and 40g mineral mix. All animal treatment ethical protocols were undertaken in accordance with animal facility ethical standard unit of Faculty of Medicine, Cairo University with the approval of Institutional Animal Ethics Committee.

Induction of Alzheimer's disease by lipopolysaccharide

The induction of Alzheimer's disease in the successive eight constructed groups of rats subjected to study treatment protocol was done by (IP) injection with 0.56 mg/ kg body weight of LPS dissolved in 1 ml of sterile PBS (pH 7) as a modified single dose [30].

2.8. Experimental design

Healthy male albino rats were divided randomly into nine equal constructed groups (eight rats each), fed on a semi purified diet for a period of

time one week prior to study initiation process. The experiment was designed as follow:

- G1 (control): Eight healthy rats acting as negative control group.
- G2 (AD): Alzheimer's disease was induced in rats by (IP) injection with 0.56 mg/kg body weight of lipopolysaccharides dissolved in 1 ml of sterile PBS as a modified single dose [30].
- G3 (AD+NaHS): LPS induced rats received NaHS (IP) at a daily single dose of 5 mg/kg dissolved in deionized water for 15 days with dose modification [28].
- G4 (AD+MSCs): LPS induced rats received MSCs intracerebrally in a single dose administration protocol at 5 μ l (5×10^5) in PBS-10% following being anesthetized with 1 ml/kg solution of 80 mg/kg/i.m of ketamine hydrochloride with dose modifications [31]. The site of rats intracerebral injection was estimated approximately half way between the eye, ear and just off the midline [32-34].
- G5 (AD+MSCs+NaHS): LPS induced rats received MSCs intracerebrally in a single dose administration protocol at 5 μ l (5×10^5) in PBS-10% with slight modifications [31] accompanied with a daily (IP) single administration of NaHS at dose of 5 mg/kg dissolved in deionized water for 15 days with dose modification [28].
- G6 (AD+kefir+GB): LPS induced rats received 4ml/kg body weight of milk kefir by oral gavage once daily for a month with slight dose modification (12,26) accompanied with concurrent single oral dose administration of 100mg/kg GB for 15 days with slight dose modification [27].
- G7:(AD+MSCs+kefir+GB):LPS induced rats received MSCs intracerebrally followed by a daily oral dose administration of kefir for a month concurrently with GB for 15 days.
- G8:(AD+NaHS+kefir+GB): LPS induced rats received NaHS (IP) at a single daily dose for

15 days followed by a daily oral dose administration of kefir for a month concurrently with GB for 15 days.

- G9:(AD+MSCs+NaHS+kefir+GB):LPS induced rats received MSCs intracerebrally with a subsequent (IP) injection of NaHS once daily for 15 days followed by a daily oral dose administration of kefir for a month concurrently with GB for 15 days.

2.9. Morris Water Maze (MWM)

After one month of applying suggested treatment protocol therapy, rats were trained to perform MWM in order to study the effect of given treatments on rat's impaired memory as a result of inducing AD which will be reflected on rat's time performance to reach the platform. Rats were placed in a circular blue pool (6 feet in diameter) filled with $26 \pm 2^\circ\text{C}$ water with a circular escape platform placed in the middle of the target quadrant 2 cm below the water surface where during training it can be exposed 1 inch above the water. Rats were subjected to four trials per day. On the fifth day, a trial performance test was undertaken with recording the time taken by each rat to reach the platform (latency time) in accordance with mental functions restoring mechanisms by the suggested administered therapy and brain tissue histopathological studies.

2.10 Blood sampling and preparation:

Following performing Morris test and before decapitation, blood was collected from the retro-orbital vein of rats, left to clot for 30 min and separated by centrifugation for 20min at 10,000 xg. Serum was kept frozen till the analysis of Caspase-3 activity level which was estimated using the ELISA technique (Cusabio Biotech,China) according to the manufacture's instruction. Animals were then anesthetized with sodium phentobarbital (60 mg/kg), killed by decapitation following brain tissue extraction.

2.11 Tissue sampling and preparation:

Brains were rapidly removed and cut into four transverse symmetrical halves by midline incision. One half was fixed in 10% formalin for

histopathological studies accompanied with MSCs labelling step using PKH26 red fluorescence cells linker kit. The other two halves were lysed in a specific designed different manners where one was prepared to perform tissue estimation of CBS relative expression using Quantitative Reverse Transcription polymerase chain reaction (QRT PCR), while the other half was prepared to perform brain tissue estimation of MAPK, Tau protein and ACAT expression by western blotting. The remaining half was left for brain tissue estimation of A β 42 level by ELISA in addition to Malondialdehyde (MDA) and Glutathione (GSH) by spectrophotometer.

2.11.1 Detection of CBS relative expression by QRT PCR:

The Purification and extraction step of total RNA from rats brain tissue using EZ-10 Spin Column Total RNA Mini-Preps kit (Bio Basic Inc., Canada) was done by tissue homogenization using RLT lysis buffer and pure ethanol followed by centrifugation for 30 sec at 12,000 xg room temperature. The extracted RNA was reverse transcribed into complementary DNA (cDNA) using a QuantiTect® Reverse Transcription Kit (QIAGEN). PCR primers were specifically designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences assigned from gene bank (Table 1). Relative expression of CBS was calculated using the comparative Ct method by $2^{-\Delta\Delta Cq}$ method. All values have been normalized to the β -actin gene and been reported as fold change over background levels detected in Alzheimer's disease.

2.11.2 Estimation of brain tissue expression of MAPK, Tau protein and ACAT by western blotting:

Brain tissue protein extraction was performed using RIPA lysis buffer (sodium chloride, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris at pH 8.0, protease and phosphatase inhibitor cocktails). The cell lysates were shaken continuously and kept 1hr in ice

prior to centrifugation at 12,000 g for 10 min 4°C. Obtained supernatants were denatured by SDS sample buffer directly by heating the protein samples for 5 min at 95°C. Estimated equal amounts of protein samples were separated by electrophoresis using a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS/PAGE) and directly been transferred onto a nitrocellulose membrane (Whatman®, Germany). After being blocked using 10% milk with TBST buffer (10 mM Tris-HCl, 120 mM NaCl, 0.1% Tween-20, pH 7.4) and kept at room temperature for 1 hr, the membranes were incubated and probed with corresponding primary antibodies (Tau Polyclonal Antibody from BioVision, p38 MAPK (D13E1) XP® Rabbit mAb from cell signalling technology and ACAT Polyclonal Antibody from BioVision) at 4°C overnight. Visualization was carried out using ECL® (plus/advanced chemi-luminescence) kit (GE healthcare, UK) as shown in figure (3C).

2.11.3 Estimation of A β 42 by ELISA in addition to Malondialdehyde (MDA) and Glutathione (GSH) by spectrophotometer.

Brain tissue was rinsed and homogenised using 1xPBS stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 xg 2-8°C. The obtained supernatant was removed and assayed immediately according to manufacturer instruction A β 1-42 (Cusabio, China), Glutathione and MDA (Biodiagnostic).

2.12 Histopathology:

Autopsy samples were taken from rat's brain tissue and fixed in 10% saline for twenty-four hours. Washing was done with deionized water followed by washing with a specified serial dilutions of alcohol (methyl and absolute ethyl alcohol) for the purpose of dehydration process. Specimens were directly cleared using xylene and placed in paraffin at 56° in sterilized oven for twenty four hours. Paraffin bees wax blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected for examination

on glass slides, deparaffinised followed by staining with haematoxylin and eosin using light electric microscope [35].

2.13 Statistical analysis

The results for eight animals per group were expressed in the form of mean ± standard error (mean± SE). One-way analysis of variance

(ANOVA) was used for the purpose of comparing variables among subjected groups. The pairwise comparisons were conducted using the Mann-Whitney U test. Obtained values at p < 0.05 were considered significant. Data were statistically analysed using the statistical package for social sciences18 software (SPSS, Chicago, IL, USA).

Table 1: Sequence of the primers used for real-time PCR Primer

CBS	Forward primer: 5' ATGCTGATCGCGCAAGAG 3' Reverse primer: 5' TCGCTCAGGAACTTGGTCAT 3'
β-actin	Forward primer :5'CCAGGCTGGATTGCAGTT3' Reverse primer: 5'GATCACGAGGTCAGGAGATG3'

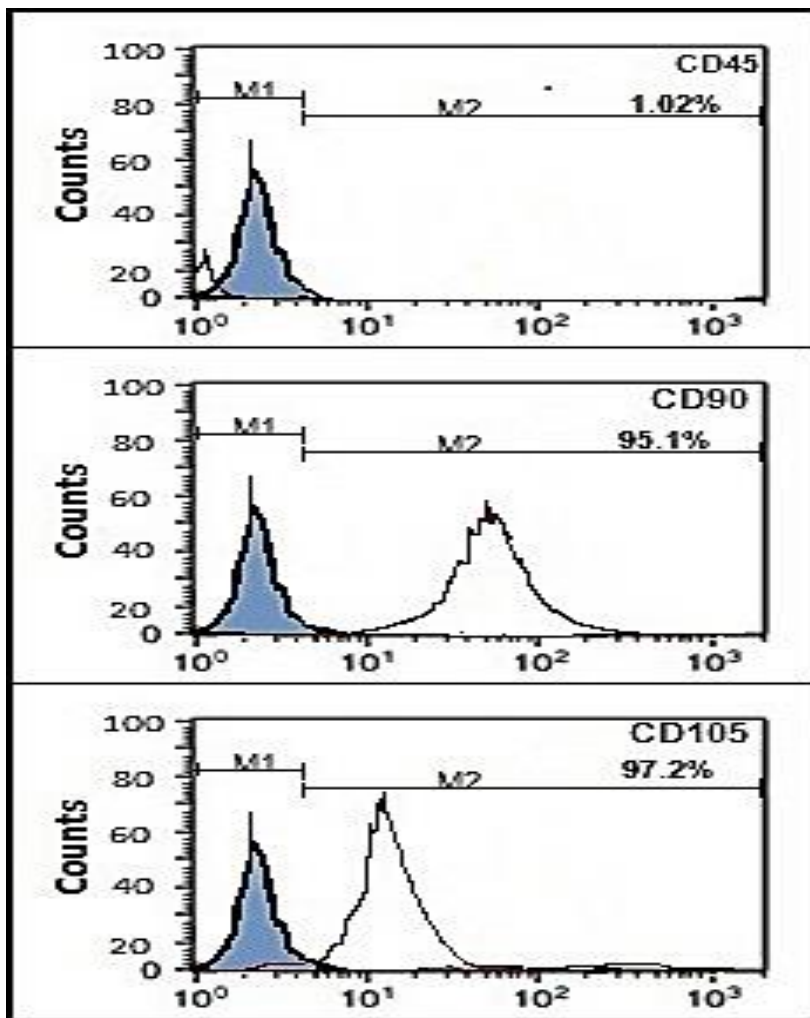


Figure 1A: Characteristics of BM-MSCs. Cells were stained with CD45-ve (A), CD90 +ve (B) and CD105+ve (C) antibody using flow cytometry.

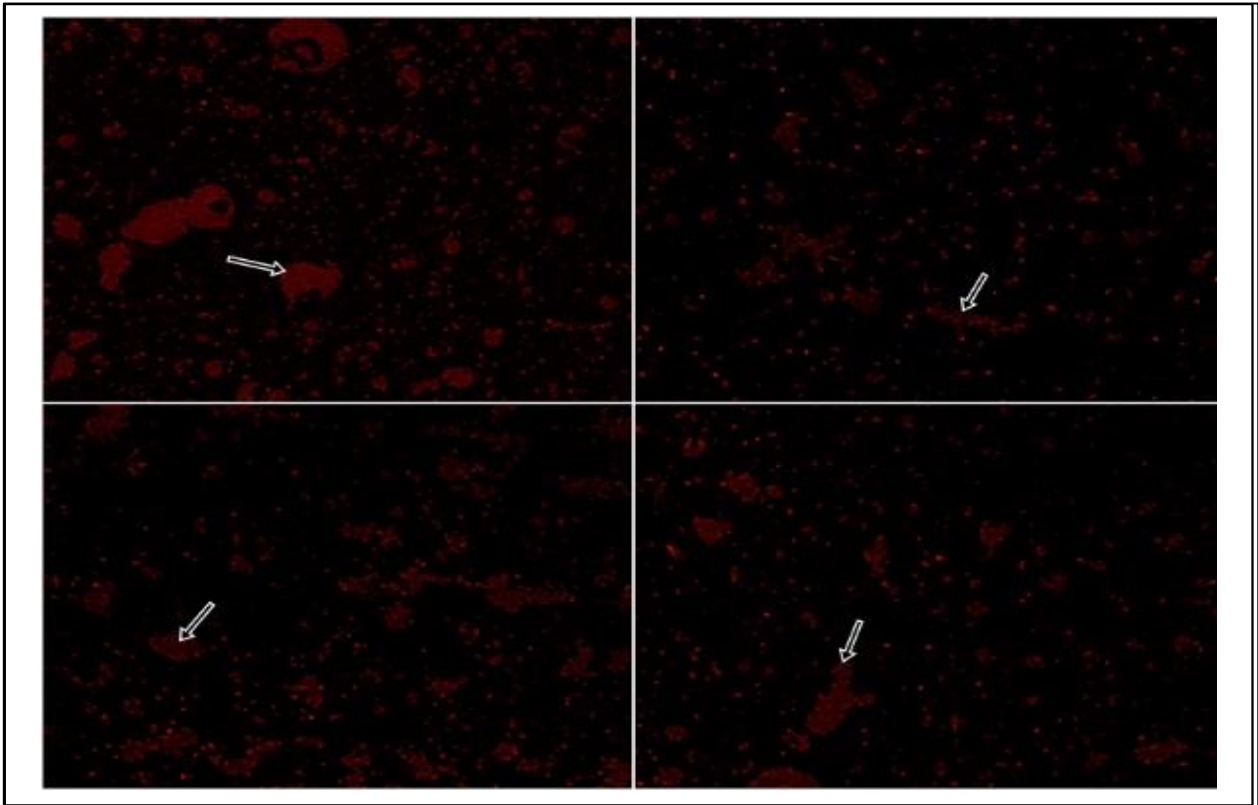


Figure 1B: Detection of MSCs labelled with PKH26 fluorescent dye in rat's brain tissue. The presence of red fluorescent (arrows) indicating homing of MSCs in brain tissue in group (G4,G5,G7&G9).

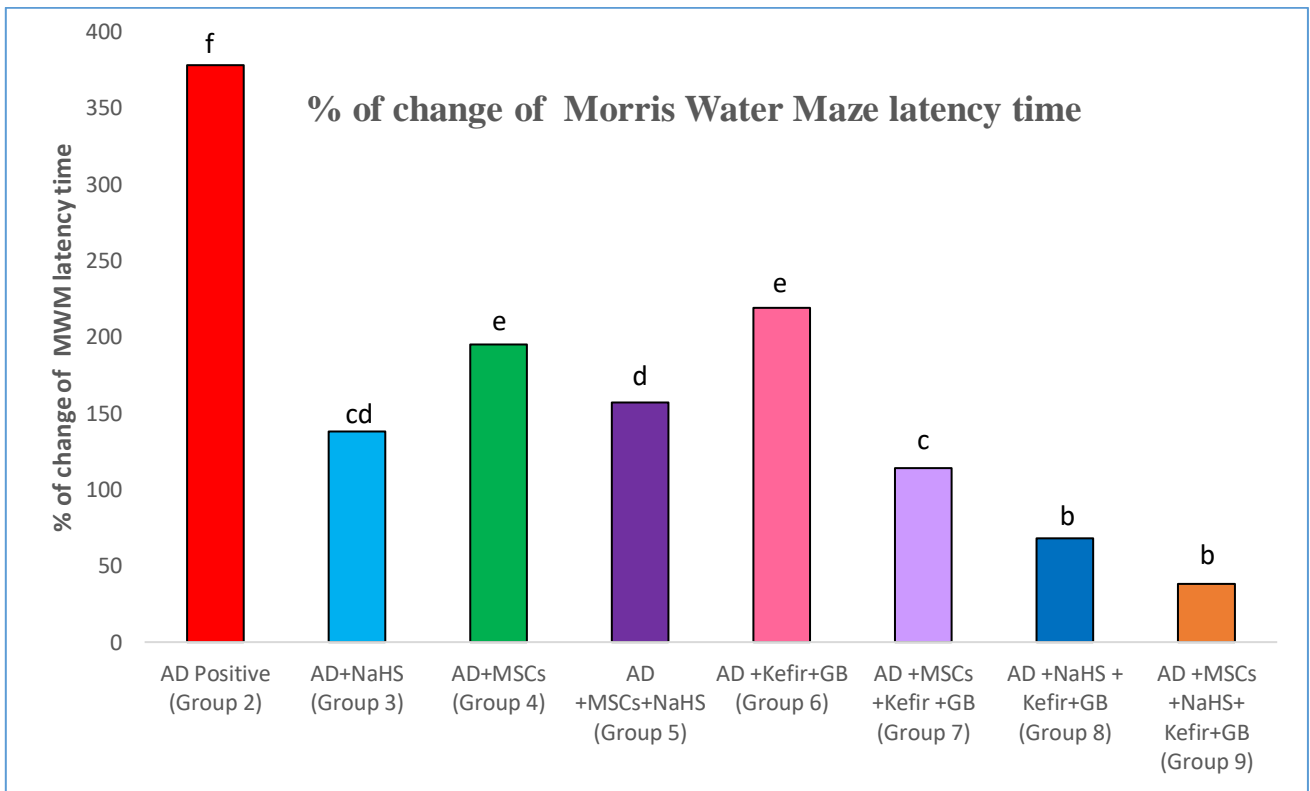


Figure 2A: Effects of NaHS, MSCs, Kefir with concurrent administration of GB and/or in an alternative manner on latency time % change consumed by rats in MWM. The same small letter means insignificant different between treated groups using Mann-Whitney U test ($P > 0.05$).

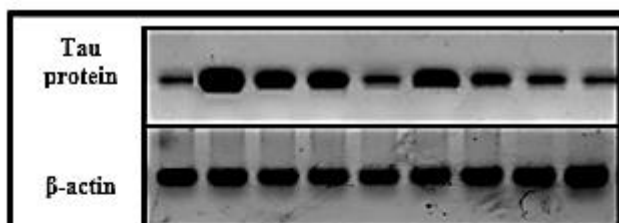


Figure 2(B): The protein expression level of Tau protein analysed by western blot for the nine successive groups against β -actin. β -actin was used as reference house-keeping gene.

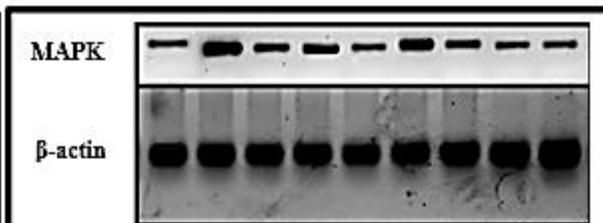


Figure 2(C): The protein expression level of MAPK protein analysed by western blot for the nine successive groups against β -actin. β -actin was used as reference house-keeping gene.

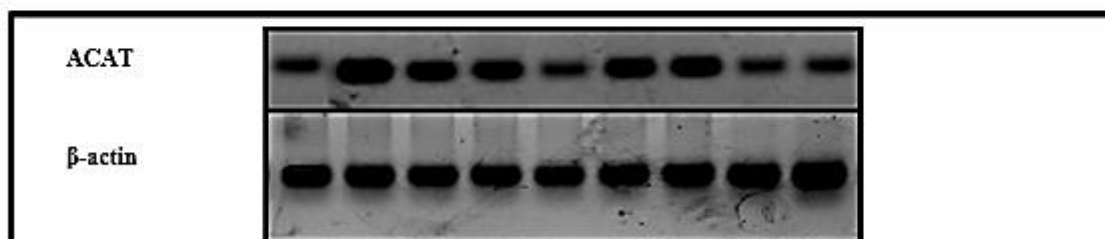


Figure 2(D): The protein expression level of ACAT protein analysed by western blot for the nine successive groups against β -actin. β -actin was used as reference house-keeping gene.

3. Results

3.1 LPS induction resulted in complete memory impairment in rats with gradual improvement following the administration of suggested medical protocol as observed by rat's performance in MWM

To determine the exact role and efficacy of the suggested therapeutic protocol in attenuating the neural cell damage and AD hallmark precipitation post to LPS administration, MWM was conducted. The obtained results reveal that LPS challenged rats spent the longest time in the conducted maze when compared with the control non-treated group and/or with the other subjected treated groups as shown in figure 2 (A). Results clearly observed the vital role of H_2S in improving the impaired memory of the LPS induced rats whether administrated alone or in simultaneous administration with MSCs and /or combination therapy of kefir and GB. The efficiency of intracerebral administration of MSCs in improving memory deterioration drawbacks was reflected on time consumed by the rats in maze when compared with control, untreated and treated groups. The concurrent administration of NaHS as an exogenous donor

of H_2S with MSCs, kefir and GB displayed the most pronounced improvement in memory functions and abilities of LPS challenged rats which can be further supported due to their synergistic actions.

3.2 Administration of NaHS and/or MSCs and/or kefir and GB decreased MAPK, Tau and ACAT brain tissue relative expression accompanied with a significant increase in CBS brain tissue level in LPS induced rats

Intraperitoneal LPS injection (IP) to rats significantly increased the expression level of tau protein, MAPK and ACAT brain relative expression accompanied with a marked decrease in CBS brain tissue expression level when compared with the negative control group (G1) as shown in figure 3(A,B,C&D). The administration of NaHS whether alone and/or intracerebral MSCs and/or combination therapy of kefir and GB significantly attenuated the expression of tau protein, MAPK and ACAT relative expression with a significant increase in CBS tissue level. The obtained results also revealed the efficacy of intracerebral administration of MSCs whether administrated alone or in combination therapy of kefir and GB

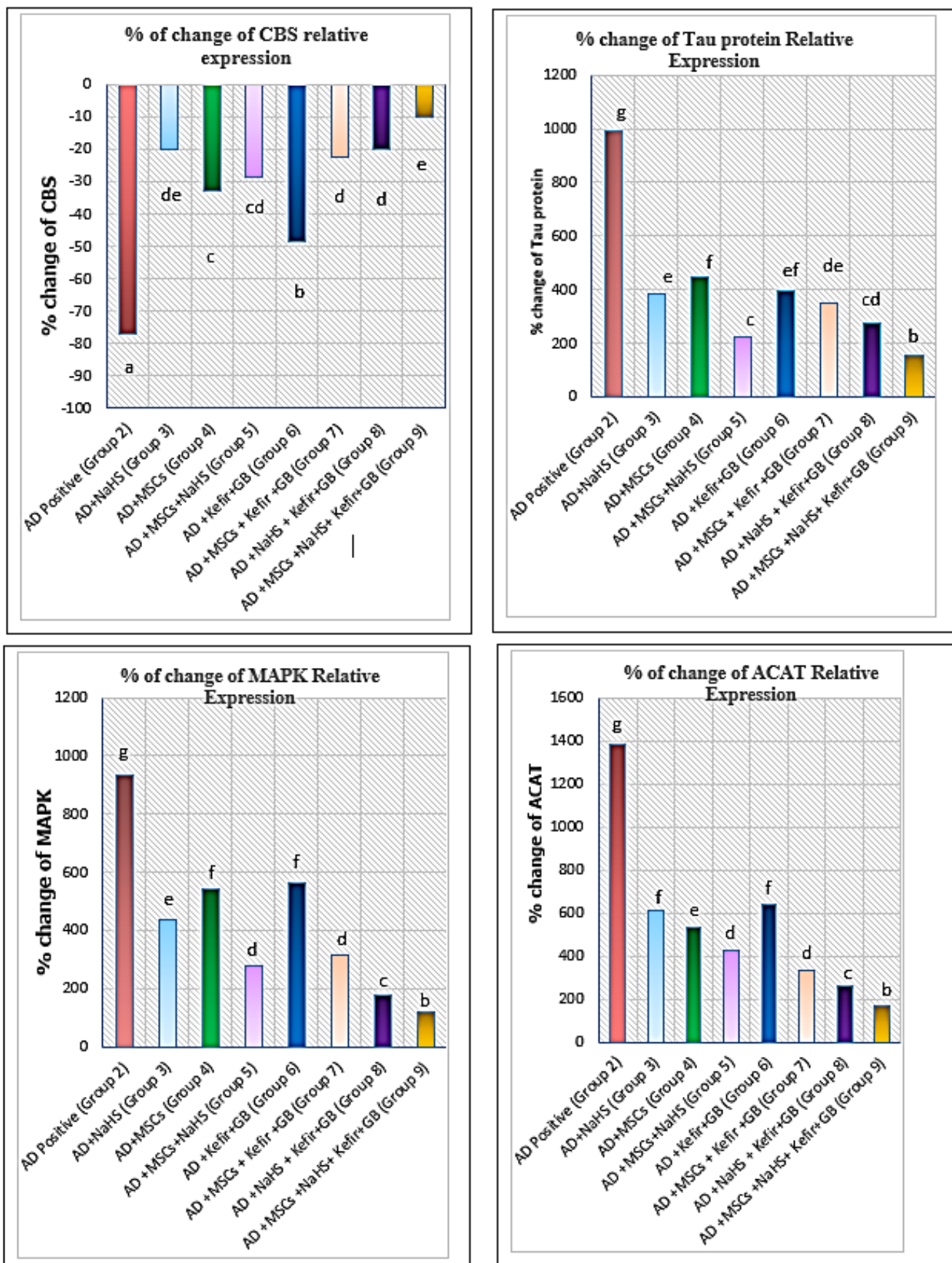


Figure (3): Effects of NaHS, MSCs, Kefir with concurrent administration of GB and/or in an alternative manner on % of change of CBS, Tau , MAPK and ACAT relative expression on LPS induced rats with respect to control (G1). The same small letter means insignificant different between treated groups using Mann-Whitney U test (P>0.05). % change corresponding to control group (G1).

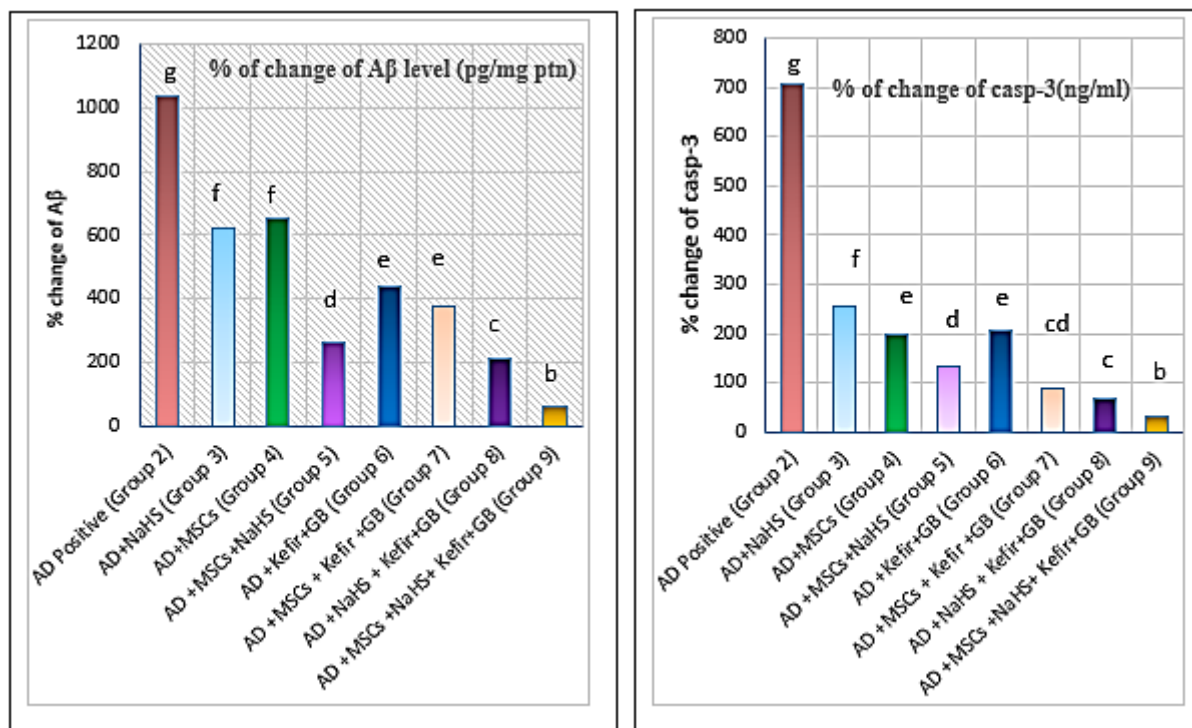


Figure 4(A): Effects of NaHS, MSCs, Kefir with concurrent administration of GB and/or in an alternative manner on % of change of Aβ1-42 (pg/mg ptn) brain tissue level and Casp-3 serum activity level (ng/ml) on LPS induced rats with respect to control (G1). The same small letter means insignificant different between treated groups using Mann-Whitney U test (P>0.05). % change corresponding to the negative control group (G1).

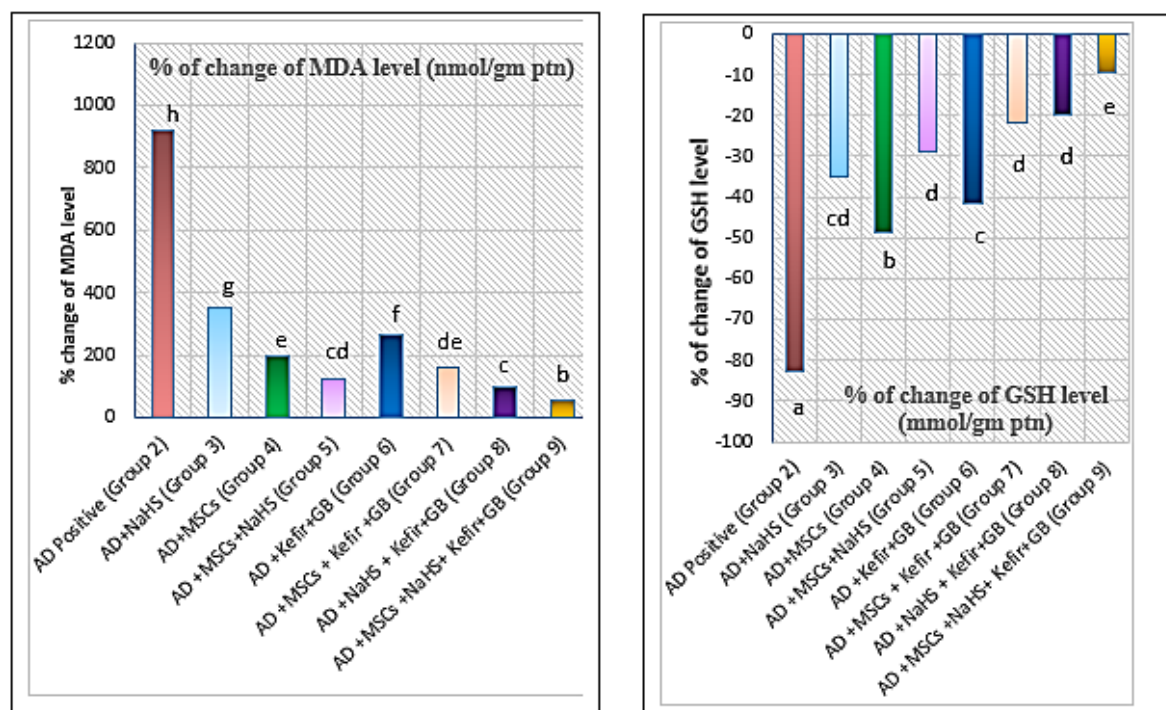


Figure 4(B&C): Effects of NaHS, MSCs, Kefir with concurrent administration of GB and/or in an alternative manner on % of change of MDA level (nmol/gm ptn) and GSH (mmol/gm ptn) brain tissue level on LPS induced rats with respect to control (G1). The same small letter means insignificant different between treated groups using Mann-Whitney U test (P>0.05). % change corresponding to the negative control (G1).

on decreasing the expression level of tau protein, MAPK and ACAT with an increase in CBS brain tissue expression level when compared with negative control group (G1) with an observed improvement following the administration of combination therapy of kefir and GB in all previously mentioned brain tissue

parameters. The concomitant co-administration of NaHS, MSCs and combination therapy of kefir and GB displayed an additive ameliorating role in decreasing the elevated level of tau protein, MAPK and ACAT with a well-marked improvement in the level of CBS relative when compared with negative control group (G1).

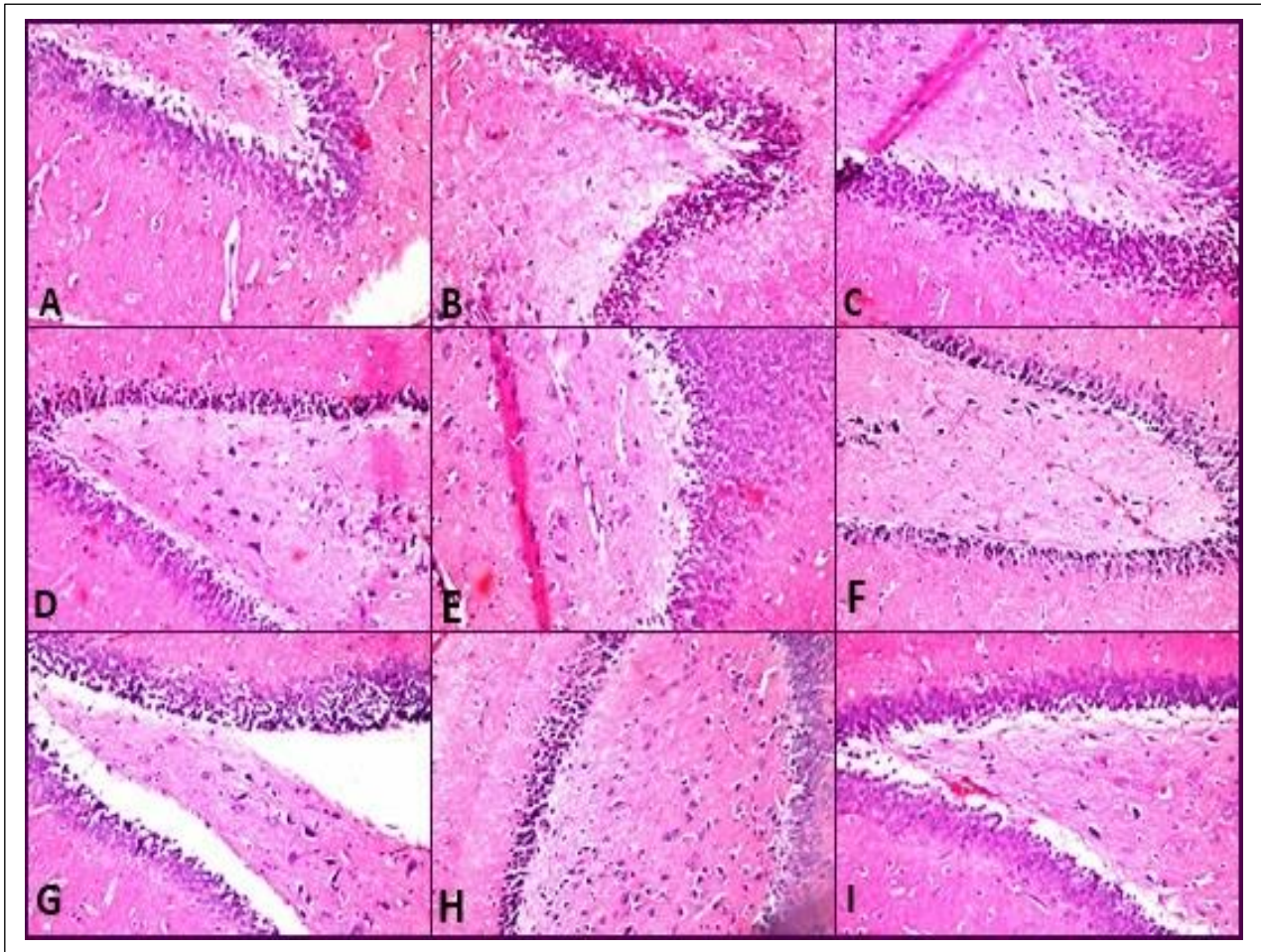


Figure 5: Histopathological examination of rat's brain tissue in: (A) negative control group (G1)(H& Ex40):showing normal histological structure of neurons in fascia dentate & hilus of hippocampus.(B): AD positive group (G2) (H&Ex40) showing nuclear pyknosis and degeneration in neurons of fascia dentate & hilus of hippocampus.(C):AD+NaHS (G3) (H&Ex40) showing nuclear pyknosis and degeneration in observed neurons of fascia dentate & hilus of hippocampus (D): AD+MSCs (G4) (H&Ex40) showing relative nuclear pyknosis and degeneration in some neurons of fascia dentate & hilus of hippocampus.(E): AD+MSCs+NaHS (G5) (H&Ex40) showing nuclear pyknosis and degeneration in few neurons of fascia dentate & hilus of hippocampus.(F): AD+Kefir and GB (G6) (H&Ex40) showing nuclear pyknosis and degeneration in neurons of fascia dentate & hilus of hippocampus.(G): AD+MSCs+Kefir and GB (G7) (H&Ex40) showing mild nuclear nuclear pyknosis and degeneration in hippocampus regions.(H): AD+NaHS+Kefir and GB (G8) (H&Ex40) showing relative nuclear nuclear pyknosis and degeneration was observed in fascia dentate & hilus of hippocampus.(I): AD+MSCs+NaHS+Kefir and GB (G9) (H&Ex40) showing relatively normal histological structures in neurons of fascia dentate and hilus of hippocampus.

3.3. Alzheimer's disease induction by LPS resulted in marked increase in amyloid- β brain tissue disposition accompanied with a marked increase in oxidative stress

Lipopolysaccharides (IP) injection resulted in a well-marked high A β level and MDA brain tissue level with a relevant decrease in GSH brain level accompanied with a significant increase in caspase-3 serum activity level with respect to the negative control group (G1). Regarding the administration of the combination therapy composed of NaHS as H₂S exogenous donor whether administrated alone and /or MSCs and /or combination therapy of kefir and GB significantly resulted in attenuating the dramatic increase in A β brain tissue precipitated level with a significant decrease in oxidative stress MDA brain level and caspase-3 serum activity level accompanied with a relevant increase in antioxidant GSH brain level in different variations where the best results were observed in G9 as shown in figure 4(A, B,C&D).

3.4. Histopathological examination of brain tissue

Regarding brain tissue histopathological findings, normal neurons histological structure of hippocampal fascia dentate and hilus was detected in rat's brain tissue (G1) as shown in figure 5(A). In LPS induced rat's brain tissue (G2), neurons of the fascia dentate and hilus of the hippocampal circuit contained nuclear pyknosis and major degenerative regions 5(B). Regarding other groups of rats subjected to the suggested treatment protocol revealed an improvement in the major histological brain tissue examination following the induction of AD by LPS but within different variations reflected by the degree of decrease in brain tissue damage and regions of nuclear pyknosis as shown in figure 5(C,D,E,F,G,H). While the most pronounced improvement was observed in group of rats treated with (MSCs+NaHS+Kefir+GB) (G9) as shown in figure 5 (I).

4. Discussion

Alzheimer's disease (AD) in addition of being a main devastating form of dementia characterized by senile plaques, tangles (NFTs), and remarkable neuron loss [36], it can be ideally described as progressive two ways reversible – irreversible of neuro-immune interactions reflexing local and systemic inflammatory disorders [37-39]. The main initiatory progression cascade of AD can be regarded to two main precipitating factors: genetic and inflammatory origin type. Regarding the inflammatory pathway, it can be initiated from LPS/TLR4 and ends with M1 microglia brain type macrophage stimulation with oxidative stress exaggerating factors release resulting in AD hallmarks brain disposition [40-45].

Accumulation of several extracellular amyloid-beta (A β) and tau protein accompanied with marked elevated MAPK expression in brain tissue were all observed in the present work after the systematic administration of LPS. These results were associated with a clearly impaired cognitive and memory functions as evidenced by MWM test indicating their contribution in AD progression in the subjected examined rats. The observed findings are in accordance with those reported by [46-48], where an imbalanced aggregated accumulation of A β 42 level in AD brain tissue was observed leading to vasoconstriction and dysregulation of vascular tone resulting in neurotoxicity, impaired blood flow within the cerebral structure, accelerated neuronal dysfunction and ended with total impaired memory function [49,50].

In the present study, the obtained increase in caspase-3 serum activity level of LPS induced rats clearly clarify the potential role of the pro-inflammatory cytokines and apoptosis in the induction of AD, activating oxidative stress and disposition of AD hallmarks in brain tissue leading to memory impairment [51,52]. Oxidative stress is one of the main triggering factors involved in AD pathology and aetiology including A β linked oligomers accumulation, tau phosphorylation, lipid dysregulation and over all

systemic inflammatory condition. Recently oxidative stress has been recognized as an important biomarker in identifying AD pathogenesis. Exaggerated oxidative stress suggested to be a result of excessive reactive oxygen species (ROS) and elevated lipid peroxidation (LPO) leading to unmaintained balance in the concentration of ions with elevated MDA brain level and well-marked decrease in the antioxidant brain GSH level [53-55].

It was observed that the expression of cystathionine beta synthase (CBS) was down-regulated in the brain tissue of LPS injected rats (G2). In accordance with our result, [59-58] reported a massive decrease in CBS relative expression in response to inflammatory cytokines resulted from AD induction by LPS with elevated MAPK expression indicating the role of CBS enzyme in down-regulating the predisposition and progression of AD in LPS induced rats. It has been also reported that S-adenosyl-L-methionine (SAM) which is a CBS activator was observed to be reduced in AD brain resulting in accumulation of homocysteine in the serum of AD patients suggesting that CBS activity is reduced in AD brains [59]. Our results also revealed, that LPS challenged rats elicited a high expression level of ACAT in brain tissue. These findings are in a good agreement with that obtained by [60], where it reflects the implication of cholesterol and cholesteryl esters in the induction and precipitation of AD. Cholesterol content in cells can affect the production of A β , by the ability of modulating cholesterol enzymes activities including secretases [60-62]. Acyl-coenzyme A: cholesterol acyl transferase (ACAT) is an endoplasmic reticulum (ER)-resident enzyme responsible for the conversion of excess free cholesterol to cholesteryl esters playing an important role in cellular cholesterol homeostasis [61,62]. Importantly, it was reported that cholesteryl ester content is significantly elevated in various brain vulnerable regions affected by AD induction whether in human or even rats leading to an increase in

membrane cholesterol level promoting the activity of amyloidogenic β -secretase and γ -secretase enzymes [60].

It has been found that H₂S level modulation in the brain has been associated with AD pathogenesis characterized by complete dysfunction and severe suppression of CBS enzyme playing a crucial role in the precipitation of AD [63-65]. Therefore the present study is undertaken to assert the crucial beneficial effects of sodium hydrogen sulphide (NaHS) as an exogenous H₂S donor on the underlying neural cell damage and over all cerebral dysfunction as a result of AD neurotoxicity through its strategic declared neuromodulatory, anti-inflammatory, anti-apoptotic, cerebral vasodilation and neuroprotective effects [63-65].

In the current study, it was also found that intra-peritoneal (IP) administration of NaHS to LPS injected rats improved the cognitive abilities of the examined rats through improving spatial memory acquisition and latency time MWM. In accordance with our obtained findings, [66,67] reported that H₂S plays an important crucial modulatory role in learning and memory functions by attenuating hippocampal damage and cognitive impairment in addition to protecting neurons against oxidative stress damage, neurotoxicity, and agitating apoptotic factors. It was also recorded that hydrogen sulphide down-regulates MAPK and tau protein expression in brain tissue of LPS induced rats by inhibiting A β production within neuron cells as a result of suppression of γ -secretase activity leading to a diminished of A β deposition and tangles formation with attenuating activated microglia and modulating marked anti-inflammatory activity induced in the brain [68-70].

Also data of the present study clearly proved the beneficial role of NaHS as exogenous H₂S donor in suppressing the increasing level of caspase-3 activity accompanied with decrease in MDA levels and simultaneous elevation in GSH in accordance with the findings of [71] who reported that H₂S exerts its neuro regenerating

effects by directly inhibiting free radicals and oxidative stress. H₂S can prevent cytokines and oxidant-induced oxidative brain tissue damage by inhibiting the expression of pro-inflammatory factors through the down-regulation of NF- κ B activation. Regarding the elevated ACAT brain tissue level in AD brain tissue, a significant decrease was observed. In Accordance with our results, [72] reported a decrease in ACAT expression and brain tissue cholesterol level as a result of CBS/H₂S overexpression enhancing the degradation and engulfing of oligomeric A β 1–42 by stimulating M1-M2 haemostasis [72-74].

Our previous study demonstrated that intravenous delivery administration of MSCs improved the neurological functions of LPS-induced rats. The goal of the current study was to evaluate whether local intracerebral transplantation of MSCs could efficiently evoke the therapeutic effect of MSCs in AD induced rats. Regarding our previous study [12], the efficacy of intravenous administration of MSCs in relieving the neurotoxicity and neuropathological disorders was demonstrated in a significant decrease in the secretion of pro-inflammatory cytokine TNF- α with up-regulation of anti-inflammatory factor IL-10. A stimulation in microglia brain type macrophage polarization towards M2 phenotype pathway which was suggested to be related to brain type neurotrophic factors secretions aiding in the survival of neural cells with a simultaneous marked decrease in bax relative expression [12]. Intracerebral injection of MSCs to LPS induced rats improved the impaired memory abilities reflecting the ability of MSCs to develop different types of neural cells to compensate damaged neural cells which was reflected on rat's performance in MWM [75]. In the present study, intracerebral transplantation of MSCs to LPS challenged rats significantly resolved amyloid beta (A β) aggregates associated with down-regulation of tau protein and MAPK brain tissue expression reflecting the ability of MSCs to decrease AD hallmarks disposition in brain

tissues which can be related to the immunomodulatory effect of MSCs. A decrease in serum Caspase-3 activity level was also observed as a result of suggested a acquired immune modulatory properties which polarize macrophages to engulf aggregated amyloid- β plaques [12,76-81].

A relevant increase in brain tissue CBS relative expression was obtained in accordance with [74,82-83] Where MSCs are vital progenitor cells owing the capability to differentiate into vital cells with maintaining tissue homeostasis and proliferation of neural stem cells. While a suppression in oxidative stress brain tissue level (MDA) with a simultaneous increase in (GSH) was observed following intracerebral MSCs transplantation. These obtained results are in accordance with [12], where oxidative stress markers in brain tissue were suggested to be suppressed by intracerebral transplantation of MSCs as a result of expressing anti-inflammatory cytokines involved in brain tissue repair introduced by MSCs via Bcl2 activation with attenuating Bax expression preventing brain damage [12,84]. An additional beneficial action of transplanting MSCs intracerebrally within LPS induced AD in rats was their ability to suppress ACAT brain tissue relative expression level, which can not only be related to the lipid engulfing macrophage polarization effect but also to its ability to suppress inflammatory cascades such as TNF- α expression and caspase-3 where they play a vital role in up regulating the brain cholesterol ACAT level [12,85-86].

To demonstrate whether single administration of MSCs intracerebrally or NaHS as exogenous H₂S sulphide donor gives a better effect on relieving AD precipitating factors to LPS induced rats or when both are given together, (G5) group was constructed. In our present study, it was found that a better results were observed in group of rats received both MSCs and NaHS. A better decrease in capase-3 serum activity level was observed in group of rats (G5) received both together as a result of the synergistic anti-

apoptotic effects of both in addition to a relevant decrease in MDA brain tissue level with a marked increase in GSH tissue accompanied with a relevant increase as well in CBS tissue expression which can also be related to the synergistic antioxidant effect of both as well. The synergistic anti-inflammatory, anti-apoptotic, immune modulatory and macrophage lipid engulfing effects of MSCs received intracerebrally and NaHS administrated IP resulted in a marked suppression in MAPK, tau and ACAT protein brain tissue expression leading to a marked suppression in amyloid beta level in brain tissue level.

In the present study the combined administration of kefir and GB significantly resolved the aggregation of A β that evidenced by the obtained decrease in A β level in brain tissue. This decrement in A β level found to be associated with significant decrease in the expression levels of tau protein and MAPK. The combined administration also significantly decreased Caspase-3 serum activity level. These findings clearly demonstrate the role of kefir and GB combination in combating the generation of AD plaques through the attenuation of amyloidogenic precipitation process. These findings are in accordance with those obtained by [87-90], who reported that GB inhibit β -secretase enzyme through direct anti-inflammatory and immune modulatory actions [87-90].

In our previous work we proved the ability of kefir to attenuate amyloid- β level in brain tissue through up regulating IL-10 and Bcl2 expression with inhibiting the release of pro-inflammatory cytokines TNF- α in brain tissue [12]. This powerful ability of kefir was also clearly proved in the current study through the attenuation of caspase-3 serum activity level with suppressing MAPK pathway accompanied with a relevant decrease in tau protein formation in the brain where these findings are in accordance with that obtained by [90], who proved the role of kefir in attenuating AD progression.

In our present study, the effect of administrating a combination medical therapy composed of kefir together with GB (G6), a decrease in brain tissue CBS relative expression was obtained where GB act as antioxidant, anti-apoptotic and anti-amyloidogenic compound by scavenging free radicals with inhibiting the activation of TLR4/NF- κ B induced by LPS through its antioxidant scavenging effect in addition to its ability to suppress BACE1 expression and TNF- α level thereby blocking the formation of the apoptosomes and apoptotic cascades leading to a progressed improvement in cognitive impairment and neuronal damage against A β toxicity [88]. While kefir seemed to exert its effect in increasing CBS level through increasing useful anti-inflammatory cytokines such as IL-10, suppression of TNF-alpha in addition to its antioxidant effect helping in regulating A β aggressive accumulation [12].

Mechanism underlying the suppression and elevation of MDA and GSH brain tissue level following the administration of kefir and GB in combination to LPS induced AD can be related to their antioxidant properties. [87], reported that GB plays an important role in combating the oxidative stress by inhibiting mitochondrial dysfunction preventing H₂O₂-inducing cell apoptosis which in turn inhibits mitochondria-mediated caspases-3 activation with simultaneous increase in the activity of PI3K/Akt pathway suppressing the release of pro inflammatory cytokines such as TNF- α and bax expression accompanied with an increase in IL-10 and bcl2 expression [87]. While the immune stimulant, anti-inflammatory and anti-oxidant activity of kefir in protecting neural tissues from oxidative stress suggested to be via decreasing the MDA brain tissue level where the bioactive peptides released during fermentation of kefir by the mean of proteolytic lactic acid bacteria which can scavenge ROS and inhibit MDA accompanied with an increase in GSH brain level [12,55].

Concerning the effect of administrating the combination therapy of kefir with GB on ACAT

brain tissue expression, a down regulation in the expression of ACAT was observed [89], reported the ability of GB to inhibit the production of A β plaques by lowering the levels of circulating free cholesterol and intracellular cholesterol levels leading to miss folding in APP processing and amyloidogenesis by its antioxidant, anti-inflammatory, immune modulatory and antiplatelet aggregation effects as well as the ability to polarize microglia to engulf excessive cholesterol due its immune modulatory effect [90]. In our previous study, we observed the ability of kefir to decrease cholesterol and lipid level by increasing the discharge of bile acids [12]. Regarding the present study, we believe that kefir has the ability to suppress ACAT which in turn decrease cholesterol ester level and intracellular cholesterol due to its oxidative scavenging, anti-inflammatory and anti-oxidant effects leading to rapid down regulation of APP neurons protein content with reducing A β production in vivo and in vitro [85-90].

Administering the combination therapy of kefir and GB together with MSCs (G7) or exogenous hydrogen sulphide donor NaHS (G8) resulted in a better progression in most subjected parameters than when receiving only Kefir and GB together as demonstrated in (G6) but with variable ratio which can be related to the dual modifying effect of the double given therapy. While it was also observed that group of rats (G9) received the suggested medical therapy composed of exogenous hydrogen sulphide donor (NAHS) with MSCs intracerebrally accompanied with kefir and GB, demonstrated the best results obtained among all other subjected groups in the present study protocol which can be related to the exaggerated synergistic anti-inflammatory, anti-apoptotic immune-modulatory and antioxidant effect which was also observed through the shortest time spent by LPS induced rats (G9) in MWM indicating the relevant progression in the previously induced memory impairment.

Concerning the histological examination of the current study, it had showed improvement in

plaques formation with severe attenuation in pyknosis, congestion and oedema within numerous brain sections including hippocampus (subiculum, fascia dentate & hilus), striatum and cerebellum following administration of suggested medical protocol to LPS induced rats in different variations and patterns revealing the efficacy of the examined materials as suggested novel therapy for AD in our study protocol.

5. Conclusion

The observed results can suggest that AD pathology seems to be an outcome of activated initiative - adaptive immune defence cascade in brain. Such initiatory inflammatory responses contribute not only to a local range of traumatic brain degenerative processes but also to an overall systemic inflammatory disorders that required to be revealed. The combination of the suggested medical strategy featuring NaHS, kefir and GB with MSCs can function as a potent neuroregenerative -modulator preventing the underlying pathological progressive hallmark brain damage cascade by protecting rats against LPS induced AD toxic aggregation with restoring brain deformities and behaviour abnormalities via attenuating and suppressing all excitatory inflammatory triggering factors. In addition to decreasing microglia M1 brain type polarization associated with exaggerated release of neuro-regenerative and BDN factors, therefore our proposed therapeutic strategy can be clinically used. Meanwhile it is recommended in order to achieve the maximum safe effect of the suggested medical protocol to be received in a quaternary manner for a longer period of time to maintain irreversible maintained memory function with complete neurons restoration and regeneration.

Conflict of interest statement

The author declared no conflict of interest.

Author contributions

MMA conducted the experiment under the supervision of LAR. OSA, NAE and BAM contributed to the experimental design, manuscript drafting and data analysis.

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Reference:

- [1] Plassman BL, Langa KM, Fisher GG, Heeringa SG, Weir DR, Ofstedal MB, et al. Prevalence of dementia in the United States: the aging, demographics, and memory study. *Neuroepidemiology* 2007;29:125-32.
- [2] Lee H, Fell J, Axmacher N. Electrical engram: how deep brain stimulation affects memory. *Trends in cognitive sciences* 2013;17:574-84.
- [3] Hardenacke K, Kuhn J, Lenartz D, Maarouf M, Mai JK, Bartsch C, et al. Stimulate or degenerate: deep brain stimulation of the nucleus basalis Meynert in Alzheimer dementia. *World neurosurgery* 2013;80:S27.e35-43.
- [4] Lyketsos CG, Targum SD, Pendergrass JC, Lozano AM. Deep brain stimulation: a novel strategy for treating Alzheimer's disease. *Innovations in clinical neuroscience* 2012;9:10-7.
- [5] Heschem S, Lim LW, Jahanshahi A, Steinbusch HW, Prickaerts J, Blokland A, et al. Deep brain stimulation of the fornix area enhances memory functions in experimental dementia: the role of stimulation parameters. *Brain stimulation* 2013;6:72-7.
- [6] Sperling RA, Laviolette PS, O'Keefe K, O'Brien J, Rentz DM, Pihlajamaki M, et al. Amyloid deposition is associated with impaired default network function in older persons without dementia. *Neuron* 2009;63:178-88.
- [7] Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. *Proc Natl Acad Sci U S A* 2001;98:676-82.
- [8] Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* 2007;55:453-62.
- [9] DiCarlo G, Wilcock D, Henderson D, Gordon M, Morgan D. Intrahippocampal LPS injections reduce Abeta load in APP+PS1 transgenic mice. *Neurobiology of aging* 2001;22:1007-12.
- [10] Savage JC, Gould DH. Determination of sulfide in brain tissue and rumen fluid by ion-interaction reversed-phase high-performance liquid chromatography. *Journal of chromatography* 1990;526:540-5.
- [11] Rosova I, Dao M, Capoccia B, Link D, Nolte JA. Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. *Stem cells (Dayton, Ohio)* 2008;26:2173-82.
- [12] Zhang X, Bian JS. Hydrogen sulfide: a neuromodulator and neuroprotectant in the central nervous system. *ACS chemical neuroscience* 2014;5:876-83.
- [13] Ali OS, Rashed LA, Badawi AM, Anwar MM. The Approaching Therapy Targeting Alzheimer's disease using Kefir grain and Mesenchymal Stem Cells *International journal of Applied Pharmaceutical and biological Research* 2016;11-21:2456-0189
- [14] Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circulation research* 2004;94:678-85.
- [15] Wu P-f, Zhang Z, Wang F, Chen J-g. Natural compounds from traditional medicinal herbs in the treatment of cerebral ischemia/reperfusion injury. *Acta pharmacologica Sinica* 2010;31:1523-31.
- [16] Greenberg DA. Neurogenesis and stroke. *CNS & neurological disorders drug targets* 2007;6:321-5.
- [17] Yu TS, Zhang G, Liebl DJ, Kernie SG. Traumatic brain injury-induced hippocampal neurogenesis requires activation of early nestin-expressing progenitors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2008;28:12901-12.
- [18] Tchantchou F, Xu Y, Wu Y, Christen Y, Luo Y. EGb 761 enhances adult hippocampal neurogenesis and phosphorylation of CREB in transgenic mouse model of Alzheimer's disease. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2007;21:2400-8.
- [19] Nada SE, Tulsulkar J, Shah ZA. Heme oxygenase 1-mediated neurogenesis is enhanced by Ginkgo biloba (EGb 761(R)) after permanent ischemic stroke in mice. *Molecular neurobiology* 2014;49:945-56.
- [20] Heurteaux C, Gandin C, Borsotto M, Widmann C, Brau F, Lhuillier M, et al. Neuroprotective and neuroproliferative activities of NeuroAid (MLC601, MLC901), a Chinese medicine, in vitro and in vivo. *Neuropharmacology* 2010;58:987-1001.
- [21] Diamond BJ, Bailey MR. Ginkgo biloba: indications, mechanisms, and safety. *The Psychiatric clinics of North America* 2013;36:73-83.
- [22] Garrote GL, Abraham AG, De Antoni GL. Chemical and microbiological characterisation of kefir grains. *The Journal of dairy research* 2001;68:639-52.
- [23] Shah NP. Functional cultures and health benefits. *International Dairy Journal* 2007;17:1262-77.
- [24] Farnworth ER. Kefir – a complex probiotic. *Food Sci Technol Bull* 2005;2:1–17.

- [25] Lopitz-Otsoa F, Rementeria A, Elguezabal N, Garaizar J. Kefir: a symbiotic yeasts-bacteria community with alleged healthy capabilities. *Revista iberoamericana de micologia* 2006;23:67-74.
- [26] Liu JR, Wang SY, Lin YY, Lin CW. Antitumor activity of milk kefir and soy milk kefir in tumor-bearing mice. *Nutrition and cancer* 2002;44:183-7.
- [27] Blecharz-Klin K, Piechal A, Joniec I, Pyrzanowska J, Widy-Tyszkiewicz E. Pharmacological and biochemical effects of Ginkgo biloba extract on learning, memory consolidation and motor activity in old rats. *Acta neurobiologiae experimentalis* 2009;69:217-31.
- [28] Xuan A, Long D, Li J, Ji W, Zhang M, Hong L, et al. Hydrogen sulfide attenuates spatial memory impairment and hippocampal neuroinflammation in beta-amyloid rat model of Alzheimer's disease. *Journal of neuroinflammation* 2012;9:202.
- [29] Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *Journal of cellular biochemistry* 1997;64:295-312.
- [30] El Sayed NS, Kassem LA, Heikal OA. Promising therapy for Alzheimer's disease targeting angiotensin converting enzyme and the cyclooxygenase-2 isoform. *Drug discoveries & therapeutics* 2009;3:307-15.
- [31] Israel Y, Ezquer F, Quintanilla ME, Morales P, Ezquer M, Herrera-Marschitz M. Intracerebral Stem Cell Administration Inhibits Relapse-like Alcohol Drinking in Rats. *Alcohol and alcoholism* 2017;52:1-4.
- [32] Liu C, Voth DW, Rodina P, Shauf LR, Gonzalez G. A comparative study of the pathogenesis of western equine and eastern equine encephalomyelitis viral infections in mice by intracerebral and subcutaneous inoculations. *The Journal of infectious diseases* 1970;122:53-63.
- [33] Pugach EK, Finley KR, Zon LI. Chapter 52 - Zebrafish and Stem Cell Research A2 - Lanza, Robert. In: Gearhart J, Hogan B, Melton D, Pedersen R, Thomas ED, Thomson J, et al., editors. *Essentials of Stem Cell Biology (Second Edition)*. San Diego: Academic Press; 2009, p. 459-66.
- [34] Shimizu S. CHAPTER 32 - Routes of Administration A2 - Hedrich, Hans J. In: Bullock G, editor *The Laboratory Mouse*. London: Academic Press; 2004, p. 527-42.
- [35] Benedikz E, Kloskowska E, Winblad B. The rat as an animal model of Alzheimer's disease. *Journal of cellular and molecular medicine* 2009;13:1034-42.
- [36] Wang X, Liu D, Huang HZ, Wang ZH, Hou TY, Yang X, et al. A Novel MicroRNA-124/PTPN1 Signal Pathway Mediates Synaptic and Memory Deficits in Alzheimer's Disease. *Biological psychiatry* 2018;83:395-405.
- [37] Fang M, Wang J, Zhang X, Geng Y, Hu Z, Rudd JA, et al. The miR-124 regulates the expression of BACE1/beta-secretase correlated with cell death in Alzheimer's disease. *Toxicology letters* 2012;209:94-105.
- [38] Lukiw WJ. Emerging amyloid beta (Ab) peptide modulators for the treatment of Alzheimer's disease (AD). *Expert opinion on emerging drugs* 2008;13:255-71.
- [39] Lukiw WJ. NF- κ B-regulated micro RNAs (miRNAs) in primary human brain cells. *Experimental neurology* 2012;235:484-90.
- [40] Paeschke N, von Haefen C, Endesfelder S, Sifringer M, Spies CD. Dexmedetomidine Prevents Lipopolysaccharide-Induced MicroRNA Expression in the Adult Rat Brain. *International journal of molecular sciences* 2017;18.
- [41] Sun Y, Qin Z, Li Q, Wan JJ, Cheng MH, Wang PY, et al. MicroRNA-124 negatively regulates LPS-induced TNF-alpha production in mouse macrophages by decreasing protein stability. *Acta pharmacologica Sinica* 2016;37:889-97.
- [42] Sun Y, Li Q, Gui H, Xu DP, Yang YL, Su DF, et al. MicroRNA-124 mediates the cholinergic anti-inflammatory action through inhibiting the production of pro-inflammatory cytokines. *Cell research* 2013;23:1270-83.
- [43] Nikolakopoulou AM, Dutta R, Chen Z, Miller RH, Trapp BD. Activated microglia enhance neurogenesis via trypsinogen secretion. *Proc Natl Acad Sci U S A* 2013;110:8714-9.
- [44] Roughton K, Andreasson U, Blomgren K, Kalm M. Lipopolysaccharide-Induced Inflammation Aggravates Irradiation-Induced Injury to the Young Mouse Brain. *Developmental Neuroscience* 2013;35:406-15.
- [45] Veremeyko T, Siddiqui S, Sotnikov I, Yung A, Ponomarev ED. IL-4/IL-13-dependent and independent expression of miR-124 and its contribution to M2 phenotype of monocytic cells in normal conditions and during allergic inflammation. *PloS one* 2013;8:e81774.
- [46] Dalrymple SA. p38 mitogen activated protein kinase as a therapeutic target for Alzheimer's disease. *Journal of molecular neuroscience : MN* 2002;19:295-9.
- [47] Marr RA, Hafez DM. Amyloid-beta and Alzheimer's disease: the role of neprilysin-2 in amyloid-beta clearance. *Front Aging Neurosci* 2014;6:187.

- [48] Lee JK, Kim NJ. Recent Advances in the Inhibition of p38 MAPK as a Potential Strategy for the Treatment of Alzheimer's Disease. *Molecules* (Basel, Switzerland) 2017;22.
- [49] Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox biology* 2018;14:450-64.
- [50] Sadigh-Eteghad S, Sabermarouf B, Majdi A, Talebi M, Farhoudi M, Mahmoudi J. Amyloid-beta: a crucial factor in Alzheimer's disease. *Medical principles and practice : international journal of the Kuwait University, Health Science Centre* 2015;24:1-10.
- [51] Alvarez S, Blanco A, Fresno M, Munoz-Fernandez MA. TNF-alpha contributes to caspase-3 independent apoptosis in neuroblastoma cells: role of NFAT. *PloS one* 2011;6:e16100.
- [52] Zhao M, Cribbs DH, Anderson AJ, Cummings BJ, Su JH, Wasserman AJ, et al. The induction of the TNFalpha death domain signaling pathway in Alzheimer's disease brain. *Neurochemical research* 2003;28:307-18.
- [53] Aybek H, Ercan F, Aslan D, Şahiner T. Determination of malondialdehyde, reduced glutathione levels and APOE4 allele frequency in late-onset Alzheimer's disease in Denizli, Turkey. *Clinical Biochemistry* 2007;40:172-6.
- [54] Rani P, Krishnan S, Rani Cathrine C. Study on Analysis of Peripheral Biomarkers for Alzheimer's Disease Diagnosis. *Frontiers in neurology* 2017;8:328.
- [55] Sunday O, Adekunle M, Temitope O, Richard A, Samuel A, Olufunminyi A, et al. Alteration in antioxidants level and lipid peroxidation of patients with neurodegenerative diseases {Alzheimer's disease and Parkinson disease}. *International Journal of Nutrition, Pharmacology, Neurological Diseases* 2014;4:146-52.
- [56] Hu LF, Wong PT, Moore PK, Bian JS. Hydrogen sulfide attenuates lipopolysaccharide-induced inflammation by inhibition of p38 mitogen-activated protein kinase in microglia. *Journal of neurochemistry* 2007;100:1121-8.
- [57] Salmina AB, Komleva YK, Szijarto IA, Gorina YV, Lopatina OL, Gertsog GE, et al. H₂S- and NO-Signaling Pathways in Alzheimer's Amyloid Vasculopathy: Synergism or Antagonism? *Frontiers in physiology* 2015;6:361.
- [58] Rose P, Moore PK, Zhu YZ. H₂S biosynthesis and catabolism: new insights from molecular studies. *Cellular and molecular life sciences : CMLS* 2017;74:1391-412.
- [59] Eto K, Ogasawara M, Umemura K, Nagai Y, Kimura H. Hydrogen Sulfide Is Produced in Response to Neuronal Excitation. *The Journal of Neuroscience* 2002;22:3386.
- [60] Shibuya Y, Chang CC, Chang TY. ACAT1/SOAT1 as a therapeutic target for Alzheimer's disease. *Future medicinal chemistry* 2015;7:2451-67.
- [61] Chang TY, Chang CC, Bryleva E, Rogers MA, Murphy SR. Neuronal cholesterol esterification by ACAT1 in Alzheimer's disease. *IUBMB life* 2010;62:261-7.
- [62] Huttunen HJ, Kovacs DM. ACAT as a drug target for Alzheimer's disease. *Neuro-degenerative diseases* 2008;5:212-4.
- [63] George AK, Behera J, Kelly KE, Zhai Y, Tyagi N. Hydrogen sulfide, endoplasmic reticulum stress and alcohol mediated neurotoxicity. *Brain research bulletin* 2017;130:251-6.
- [64] Gao C, Chang P, Yang L, Wang Y, Zhu S, Shan H, et al. Neuroprotective effects of hydrogen sulfide on sodium azide-induced oxidative stress in PC12 cells. *International journal of molecular medicine* 2018;41(1):242-50.
- [65] Zhang JY, Ding YP, Wang Z, Kong Y, Gao R, Chen G. Hydrogen sulfide therapy in brain diseases: from bench to bedside. *Medical gas research* 2017;7:113-9.
- [66] Li G, Yang Y, Dong HJ, Lin L. The research progress of mesenchymal stem cells in the treatment of Traumatic brain injury. *Turkish neurosurgery* 2017;10:5137-5149
- [67] Zhang M, Shan H, Chang P, Wang T, Dong W, Chen X, et al. Hydrogen sulfide offers neuroprotection on traumatic brain injury in parallel with reduced apoptosis and autophagy in mice. *PloS one* 2014;9:e87241.
- [68] Nagpure BV, Bian J-S. Hydrogen Sulfide Inhibits A2A Adenosine Receptor Agonist Induced β -Amyloid Production in SH-SY5Y Neuroblastoma Cells via a cAMP Dependent Pathway. *PloS one* 2014;9:e88508.
- [69] Nogueira JE, Soriano RN, Fernandez RA, Francescato HD, Saia RS, Coimbra TM, et al. Effect of Physical Exercise on the Febrigenic Signaling is Modulated by Preoptic Hydrogen Sulfide Production. *PloS one* 2017;12:e0170468.
- [70] Rosario-Alomar MF, Quinones-Ruiz T, Kurouski D, Sereda V, Ferreira EB, Jesus-Kim LD, et al. Hydrogen sulfide inhibits amyloid formation. *The journal of physical chemistry B* 2015;119:1265-74.
- [71] Zhang Q, Lu Y, Bian H, Guo L, Zhu H. Activation of the $\alpha 7$ nicotinic receptor promotes lipopolysaccharide-induced conversion of M1

microglia to M2. American Journal of Translational Research 2017;9:971-85.

[72] Cheung SH, Kwok WK, To KF, Lau JY. Anti-atherogenic effect of hydrogen sulfide by over-expression of cystathionine gamma-lyase (CSE) gene. PloS one 2014;9:e113038.

[73] Mani S, Li H, Untereiner A, Wu L, Yang G, Austin RC, et al. Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. Circulation 2013;127:2523-34.

[74] Zhao Y, Wei H, Kong G, Shim W, Zhang G. Hydrogen sulfide augments the proliferation and survival of human induced pluripotent stem cell-derived mesenchymal stromal cells through inhibition of BKCα. Cytotherapy 2013;15:1395-405.

[75] Babaei P, Soltani Tehrani B, Alizadeh A. Transplanted Bone Marrow Mesenchymal Stem Cells Improve Memory in Rat Models of Alzheimer's Disease. Stem Cells International 2012;2012:8.

[76] Amemori T, Jendelova P, Ruzicka J, Urdzikova LM, Sykova E. Alzheimer's Disease: Mechanism and Approach to Cell Therapy. International journal of molecular sciences 2015;16:26417-51.

[77] Gu W, Song L, Li XM, Wang D, Guo XJ, Xu WG. Mesenchymal stem cells alleviate airway inflammation and emphysema in COPD through down-regulation of cyclooxygenase-2 via p38 and ERK MAPK pathways. Scientific reports 2015;5:8733.

[78] Naaldijk Y, Jager C, Fabian C, Leovsky C, Blucher A, Rudolph L, et al. Effect of systemic transplantation of bone marrow-derived mesenchymal stem cells on neuropathology markers in APP/PS1 Alzheimer mice. Neuropathology and applied neurobiology 2017;43:299-314.

[79] Neirinckx V, Coste C, Rogister B, Wislet-Gendebien S. Concise review: adult mesenchymal stem cells, adult neural crest stem cells, and therapy of neurological pathologies: a state of play. Stem cells translational medicine 2013;2:284-96.

[80] Turgeman G. The therapeutic potential of mesenchymal stem cells in Alzheimer's disease: converging mechanisms. Neural Regen Res 2015;10:698-9.

[81] Yun HM, Kim HS, Park KR, Shin JM, Kang AR, il Lee K, et al. Placenta-derived mesenchymal stem cells improve memory dysfunction in an Abeta1-42-

infused mouse model of Alzheimer's disease. Cell death & disease 2013;4:e958.

[82] Li C, Guo Z, Guo B, Xie Y, Yang J, Wang A. Inhibition of the endogenous CSE/H(2)S system contributes to hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cells. Molecular medicine reports 2014;9:2467-72.

[83] Yang R, Liu Y, Shi S. Hydrogen Sulfide Regulates Homeostasis of Mesenchymal Stem Cells and Regulatory T Cells. Journal of dental research 2016;95:1445-51.

[84] Calio ML, Marinho DS, Ko GM, Ribeiro RR, Carbonel AF, Oyama LM, et al. Transplantation of bone marrow mesenchymal stem cells decreases oxidative stress, apoptosis, and hippocampal damage in brain of a spontaneous stroke model. Free radical biology & medicine 2014;70:141-54.

[85] Abdel Aziza MT, Atta HM, Samer H, Ahmed HH, Rashed LA, Sabry D, et al. Heme oxygenase effect on mesenchymal stem cells action on experimental Alzheimer's disease. EXCLI journal 2013;12:778-92.

[86] Frodermann V, van Duijn J, van Pel M, van Santbrink PJ, Bot I, Kuiper J, et al. Mesenchymal Stem Cells Reduce Murine Atherosclerosis Development. Scientific reports 2015;5:15559.

[87] Giuliani D, Ottani A, Zaffe D, Galantucci M, Strinati F, Lodi R, et al. Hydrogen sulfide slows down progression of experimental Alzheimer's disease by targeting multiple pathophysiological mechanisms. Neurobiol Learn Mem 2013;104:82-91.

[88] Luo Y, Smith JV, Paramasivam V, Burdick A, Curry KJ, Buford JP, et al. Inhibition of amyloid-beta aggregation and caspase-3 activation by the Ginkgo biloba extract EGb761. Proc Natl Acad Sci U S A 2002;99:12197-202.

[89] Rhein V, Giese M, Baysang G, Meier F, Rao S, Schulz KL, et al. Ginkgo biloba extract ameliorates oxidative phosphorylation performance and rescues abeta-induced failure. PloS one 2010;5:e12359.

[90] Wu Y, Wu Z, Butko P, Christen Y, Lambert MP, Klein WL, et al. Amyloid-beta-induced pathological behaviors are suppressed by Ginkgo biloba extract EGb 761 and ginkgolides in transgenic Caenorhabditis elegans. The Journal of neuroscience : the official journal of the Society for Neuroscience 2006;26:13102-13.

