The Anti-Apoptotic Mechanism of Action of a Water Soluble Curcumin Derivative and Mscs in Alzheimer Disease Induced In Rats

MT Abdel Aziz[a], Ameen Rizk [a],HH Ahmed[a], LA Rashed[a], M Sohier[b], Ahmed Talaat [a] and MA ALKaffas[a]

[a]Department of Medical Biochemistry, Unit of Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt

[b]Department of pathology, Faculty of Medicine, Cairo University, Cairo, Egypt

ABSTRACT

Objective: *The objective is to evaluate the possible mechanisms of action of a water soluble curcumin derivative and MSCs in Alzheimer disease (AD).* ***Materials and Methods:*** *This work included: Seventy rats which were divided equally into: control group, AD group (induced by lipopolysaccharide), AD group received a novel curcumin derivative (NCD), AD group received pure curcumin, AD group received MSCs then NCD, AD group received MSCs, AD group received MSCs with NCD. Histopathological examination and gene expression of Bax, bcl2 and Seladin-1 gene expression in brain tissue and estimation of MDA and GSH in brain tissue by colorimetry were performed.* ***Results:*** *Histopathological examination of brain tissue from AD animals revealed the presence of plaques. Administration of MSCs or MSCs with NCD into rats after induction of experimental AD improved the histopathological picture with plaque disappearance and improved the behavior study, while other groups showed decrease of plaque formation. Gene expression demonstrated that bcl2 and Seladin-1 were upregulated while Bax gene was downregulated in all treated groups with a significant effect in the group treated with MSCs alone and group treated with both MSCs and NCD. MDA levels were decreased while GSH levels were increased in all treated groups.* ***Conclusion:*** *Administration of BM-derived MSCs either alone or with the NCD exerts a therapeutic effect on the brain lesion in Alzheimer's disease. This effect may be through the antioxidant and antiapoptotic action of both MSCs and curcumin. These data suggests that MSCs and curcumin may be a therapeutic target in treatment of AD.*

Key words: *Alzheimer disease, Mesenchymal stem cells, Novel curcumin derivative.*

INTRODUCTION

Alzheimer's disease is a progressive, neurodegenerative disease characterized in the brain by abnormal clumps (amyloid plaques) and tangled bundles of fibers (neurofibrillary tangles) composed of misplaced proteins. It manifests by a progressive decline in cognitive abilities such as memory, comprehension and language expression [(1).]

Mesenchymal stem cells (MSCs) are pluripotent cells isolated from the bone marrow and other various organs. They are able to proliferate and self-renew, as well as to give rise to progeny of at least the osteogenic and adipogenic lineages [(2).]

In a previous work**,** it has been found that infusion of BM-derived MSCs either alone or associated with heme-oxygenase (HO) inducer exert a therapeutic effect against the brain lesion in Alzheimer's disease possibly through decreasing the brain cholesterol level and increasing seladin-1 gene expression [(3).]

Curcumin (1,7bis (4 hydroxy-3 methoxy phenol)-1, 6 heptadiene-3, 5 dione) is a yellow phenolic compound present in turmeric (Curcuma-longa) a widely used spice in Indian cuisine. Curcumin has a number of biological applications along with a significant, anti-inflammatory antioxidant activity both invivo and invitro [(4).]

Studies over the past three decades related to absorption, distribution, metabolism and excretion of curcumin have revealed poor absorption and rapid metabolism of curcumin that severely curtails its bioavailability [(5).] Because of the poor bioavailability of pure curcumin, a new water soluble curcumin derivative (Patent pending PCT/EG2010/000008) was used in this study.

This work aims to study the possible mechanisms of action of a water soluble curcumin derivative and MSCs in Alzheimer disease.

MATERIALS & METHODS

This work was performed at the Unit of Biochemistry and Molecular Biology at The Medical Biochemistry Department, Faculty of Medicine, Cairo University, Cairo, Egypt.

A novel water soluble curcumin derivative (NCD) was developed through covalent modification of the curcumin molecule on sites remote from its natural functional groups. The NCD was presented free of charge to the participating researchers as a personal non-profit scientific participation in the present study. The novel derivative with 53.2 % curcumin content is registered as international patent protected by the rights of "The Patent Cooperation Treaty" and is the personal property of its inventors (PCT/EG2008/000044, WO 2010/057503, Regional phase European Patent Application No. 08878223) [(6).]

Preparation of the animal model

Experimental animals

This study included seventy female rats obtained from an inbred strain of matched age and weight (6 months-1 year & 120-150gm). Animals were inbred in the Experimental Animal Unit, Faculty of Medicine, Cairo University. Rats were bred and maintained in an air conditioned animal house with specific pathogen free conditions, and were subjected to a 12:12-h daylight/darkness. Animals were fed a semi-purified diet that contained (gm/kg): 200 casein, 555 sucrose, 100 cellulose, 100 fat blends, 35 vitamin mix, and 35 mineral mix. All animal experiments received approval from the Institutional Animal Care Committee. They were divided into 7 groups as follow:

Group 1 (AD): Ten rats as a positive control group (induced by Lipopolysaccharide (LPS) that was

given as a single injection at a dose of 0.8mg/kg, intraperitoneally every week for 3 weeks) [7].

Group 2 (control): Ten rats as a negative control group (normal healthy rats).

Group 3 (NCD): Ten induced Alzheimer rats that received curcumin derivative only (150 mg/kg, body weight, orally daily for 3 weeks).

Group 4 (Pure): Ten induced Alzheimer rats that received pure curcumin (80 mg/kg, body weight, orally daily for 3 weeks) [8].

Group 5 (MSCs then NCD): Ten induced Alzheimer rats that received MSCs (which were processed and cultured for 14 days, in a single dose of 106 cells per rat[9], given by Intravenous infusion at the rat tail vein for one Month),then NCD(150 mg/kg, body weight, orally daily for 3 weeks) two weeks later.

Group 6 (MSCs): Ten induced Alzheimer rats that received intraperitoneal MSCs only.

Group 7 (MSCs+NCD): Ten induced Alzheimer rats that received MSCs (which were processed and cultured for 14 days, in a single dose of 10^6 cells per rat[9], given by Intravenous infusion at the rat tail vein for one Month)and NCD (150 mg/kg, body weight, orally daily for 3 weeks).

At the planned time (30 days), Animals were sacrificed by anesthesia, and brain tissue was harvested for assessment of the following:

1. Real time qPCR for Bax, bcl2 and Seladin-1 gene expression in brain tissue.
2. Estimation of Malondialdehyde (MDA) and Reduced glutathione (GSH) in rat brain by colorimetry.
3. Histopathological examination of brain tissues.

I. Isolation, propagation, identification and labeling of BM-derived MSCs from rat

A) Isolation and propagation of BM-derived MSCs from rats

Bone marrow was harvested by flushing the tibiae and femurs of 6-week-old male white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10 % fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1 % penicillin–streptomycin (GIBCO/BRL). Cells were incubated at 37 °C in 5 % humidified CO_2 for 12–14 days as primary culture or upon formation of large colonies. When large colonies developed (80–90 % confluence), cultures were washed twice with phosphate buffer saline (PBS) and the cells were trypsinized with 0.25 % trypsin in 1 mM EDTA (GIBCO/BRL) for 5 min at 37 °C. After centrifugation, cells were resuspended in serum-supplemented medium and incubated in 50 cm^2 culture flask (Falcon). The resulting cultures were referred to as first-passage cultures[10]. MSCs in culture were characterized by their adhesiveness and fusiform shape.

B) Identification of BM- derived MSCs from rat

Cells were identified as being MSCs by their morphology, adherence, and their typical fusiform appearance [11] .CD29 gene expression was detected by RT-PCR as a marker of MSCs [3].

C) Labeling of MSCs with PKH26

MSCs were harvested during the 4th passage and were labeled with PKH26, which is a red fluorochrome. The fluorochrome has excitation (551 nm) and emission (567 nm) characteristics compatible with rhodamine or phycoerythrin detection systems. The linkers are physiologically stable and show little to no toxic side-effects on cell systems. Labeled cells retain both biological and proliferating activity, and are ideal for *in vitro* cell labeling, *in vitro* proliferation studies and long, *in vivo* cell tracking. In the current work, MSCs were labeled with PKH26 from Sigma Company (St. Louis, MO/USA). Cells were centrifuged and washed twice in serum free medium. Cells were pelleted and suspended in dye solution. Cells were injected intravenously into rat tail vein. After one month, brain tissue was examined with a fluorescence microscope to detect and trace the cells.

II -QRT PCR for Bax, bcl2 and Seladin-1 gene expression in brain tissue:

Total RNA was extracted from brain tissue homogenate using RNeasy purification reagent (Qiagen, Valencia, CA). cDNA was generated from 5 µg of total RNA extracted with 1 µl (20 pmol) antisense primer and 0.8 µl superscript AMV reverse transcriptase for 60 min at 37 °C.

The relative abundance of mRNA species was assessed on an ABI prism 7500 sequence detector system (Applied Biosystems, Foster City, CA). PCR primers were designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences from GenBank (Table 1). All primer sets had a calculated annealing temperature of 60°. Quantitative RT-PCR was performed in duplicate in a 25-µl reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer and 2–3 µl of cDNA. Amplification conditions were 2 min at 50°, 10 min at 95° and 40 cycles of denaturation for 15 s and annealing/extension at 60° for 10 min. Data from real-time assays were calculated using the v1·7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of Bax, bcl_2, and seladin-1 mRNA was calculated using the comparative Ct method as previously described. All values were normalized to the □-actin gene and reported as fold change over background levels detected in Alzheimer Disease.

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

Bax	Forward primer: ’°GTTGCCCTCTTCTACTTTG ’3 Reverse primer: ’°AGCCACCCTGGTCTTG’3
Bcl2	Forward 5′ CGGGAGAACAGGGTATGA 3′ Reverse 5′ CAGGCTGGAAGGAGAAGAT 3′
Seladin-1	Forward primer: ’°ATCGCAGCTTTGTGCGATG’3 Reverse primer: ’°CACCAGGAAACCCAGCGT’3
β-actin	Forward primer :’°CCAGGCTGGATTGCAGTT’3 Reverse primer: ’°GATCACGAGGTCAGGAGATG’3

III-Estimation of the levels of MDA and GSH in brain tissue by colorimetry:

Brain tissue MDA was assayed by a commercial kit supplied by Biodiagnostic, Egypt [(12)]. Brain tissue GSH was assayed by a commercial kit supplied by Biodiagnostic, Egypt [(13)].

IV-Histopathological examination of brain tissues

Autopsy samples were taken from the brain of rats in different groups and fixed in 10% formol saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin &eosin stain for routine examination then examination was done through the light electric microscope [(14)].

Statistical analysis

Data were coded and entered using the statistical package SPSS version 16. Data was presented as mean ± standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while non para metrical kruscal-wallis test and mann-whitney test were used for non-normally distributed quantitative variables. P-values less than 0.05 were considered statistically significant.

RESULTS

I- MSCs culture, identification & homing

Isolated and cultured undifferentiated MSCs reached 70-80 % confluence in 14 days. Typical fusiform appearance in culture **(Figure 1)**.MSCs labeled with PKH26 fluorescent dye were detected in the brain tissues confirming that these cells homed into the brain tissues **(Figure 3).** In addition MSCs were identified by surface marker CD29 (+) by RT-PCR as a marker of MSCs **(Figure2).**

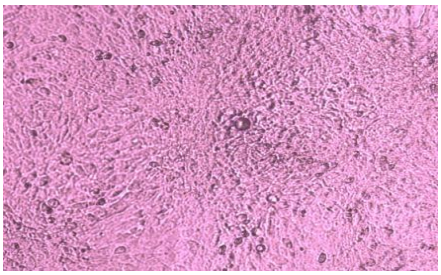
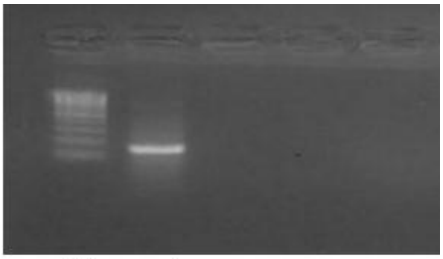


Figure (1): MSCs in culture.



M 1

Figure (2): An agarose gel electrophoresis shows PCR products of CD29 gene expression in MSC culture (261 bp) (as a molecular marker for rat MSCs)

Lane M: DNA marker with 100 bp.

Lane 1: MSCs culture.

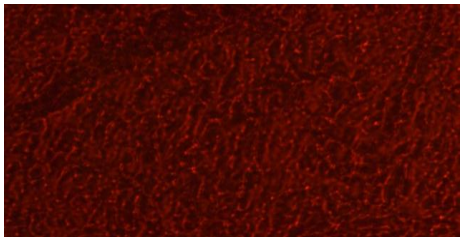


Figure (3): Labeling of MSCs with PKH26 fluorescent dye detected in the brain tissue, confirming that these cells homed into the brain tissue.

II-Gene expression of Bax, bcl2 and Seladin-1 in brain tissue (table 2)

- Our results showed a significant ($p<0.001$) decrease in the mean Bcl_2 and Seladin-1 gene expression and a significant ($p<0.001$) increase in the mean Bax gene expression in the AD group compared to the control group.
- Treatment with NCD, MSCs, MSCs followed by NCD or MSCs +NCD significantly increased the gene expression of Bcl2 and seladin-1 and significantly decreased the mean gene expression of Bax compared to the AD group, while administration of pure curcumin decreased the gene expression of Bax, when compared to the AD group.
- On comparing the effect of NCD and pure curcumin on gene expression of the previously mentioned genes, NCD provided more effective results than pure curcumin except for seladin-1.
- Our results reported that when AD rats received MSCs, there was a significant increase in seladin -1gene expression compared to the AD rats who received the oral NCD.
- On the other hand we reported that the concomitant injection of MSCs and oral administration of NCD or the injection of MSCs followed 2 weeks later by NCD to AD rats showed a non significant difference when compared to the AD rats injected with MSCs, except for Bcl_2 where the concomitant injection of MSCs and oral administration of NCD showed significantly increased Bcl_2 gene expression when compared to the AD rats injected with MSCs and the injection of MSCs followed 2 weeks later by NCD.
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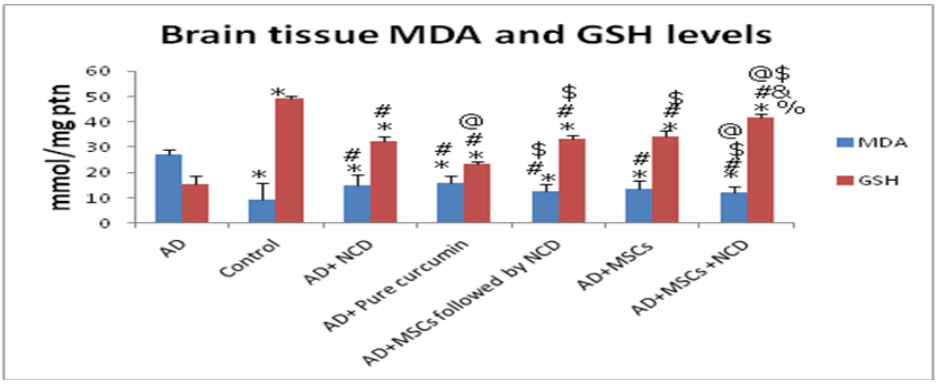
Table (2): Relative gene expression of Bax, Bcl_2 and Seladin-1 in rat brain in studied groups.

Genes	AD	Control	AD + NCD	AD + Pure curcumin	AD+MSCs followed by NCD (2 weeks later)	AD + MSCs	AD+MSCs + NCD
Bax Mean± SD	$2.23^{(a)} \pm 0.34$	$0.16^{(b)} \pm 0.05$	$0.85^{(c)} \pm 0.17$	$1.12^{(d)} \pm 0.23$	$0.51^{(e)} \pm 0.17$	$0.65^{(c)(e)(f)} \pm 0.12$	$0.44^{(e)(g)} \pm 0.08$
Bcl_2 Mean± SD	$9.26^{(a)} \pm 1.22$	$26.66^{(b)} \pm 1.7$	$14.41^{(c)} \pm 1.59$	$11.1^{(a)} \pm 0.96$	$18.25^{(d)} \pm 2.11$	$17.72^{(d)(e)} \pm 1.18$	$22.9^{(f)} \pm 3.04$
Seladin-1 Mean± SD	$0.22^{(a)} \pm 0.02$	$1.8^{(b)} \pm 0.25$	$0.68^{(c)} \pm 0.11$	$0.53^{(c)(d)} \pm 0.16$	$0.86^{(c)(e)(f)} \pm 0.06$	$1^{(f)} \pm 0.21$	$1.24^{(g)(f)} \pm 0.28$

Values not sharing a common superscript differ significantly ($p < 0.05$).

III-Estimation of MDA and GSH by colorimetry (Figure 4)

- Our results showed a significant ($p<0.001$) increase in the mean MDA level (27.08±3.27) and a significant ($p<0.001$) decrease in the mean GSH level in the AD group (15.38±2.05) compared to the control groups (9.36±0.92 and 49.32±6.63), respectively.
- On comparing the effect of oral administration of NCD and pure curcumin on MDA and GSH levels in the AD, NCD significantly increased ($p<0.001$) the GSH level compared to the pure curcumin while no significant difference ($p= 0.960$) was detected in MDA levels.
- The administration of MSCs to the AD group resulted in a nonsignificant difference ($p=0.603$) in the levels of MDA and GSH ($p=.879$) compared to the AD group receiving the NCD.
- Our results showed that the concomitant injection of MSCs and oral administration of NCD or the injection of MSCs followed 2 weeks later by NCD to AD rats showed a non significant difference ($p=.999$ and $p=0.558$),respectively in MDA levels when compared to the AD rats injected with MSCs. On the other hand, the mean GSH level in the AD group receiving MSCs +NCD (41.51±2.26) showed a significant increase ($p<0.05$) when compared to the AD group receiving MSCs (34.37±3.49) and to the AD group receiving MSCs then NCD (33.42±3.25).

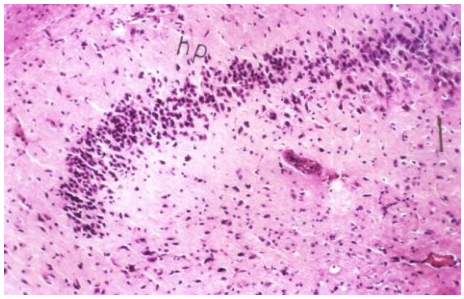


* statistically significant compared to corresponding value in AD group (P<0.05)
\# statistically significant compared to corresponding value in control group (P<0.05)
@statistically significant compared to corresponding value in NCD group (P<0.05)
$ statistically significant compared to corresponding value in pure curcumin group (P<0.05)
& statistically significant compared to corresponding value in MSCs then NCD group (P<0.05)
% statistically significant compared to corresponding value in MSCs group (P<0.05)

Figure (4): Comparison between the MDA and GSH levels in rat brain of the studied groups.

VI-Histopathological examination of brain tissues in different groups

- Histopathological results of rat brain control group showed intact histological structure of the hippocampus **(Figure 5).**
- Histopathological examination of the brain tissue of the **AD group** showed focal areas of plaque formation and gliosis in the striatum of the cerebrum **(Figure 6). AD received NCD group:** Focal areas of plaque formation with diffuse gliosis in between **(Figure 7), AD received pure curcumin group:** Focal areas of plaque formation in the striatum of the cerebrum **(Figure 8).AD received MSCs then NCD group:** The striatum of the cerebrum showed focal plaques formation **(Figure 9), AD received MSCs group:** Focal hemorrhage was detected in the hippocampus **(Figure 10). AD received MSCs and NCD group:** Mild diffuse gliosis was detected in the hippocampus **(Figure 11).**



Hp=hippocampus H&E (X40)

Figure (5): Histopathological examination of control group

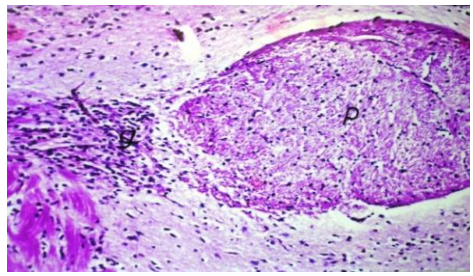


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Figure (6): Histopathological examination of rat brain of induced Alzheimer model.

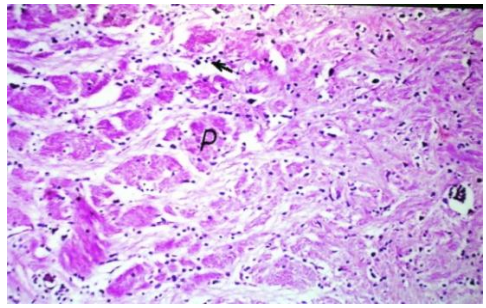

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Figure (7): Histopathological examination of rat brain of AD that received NCD.

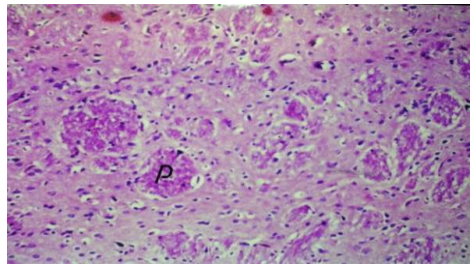

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Figure (8): Histopathological examination of rat brain of AD that received pure curcumin .

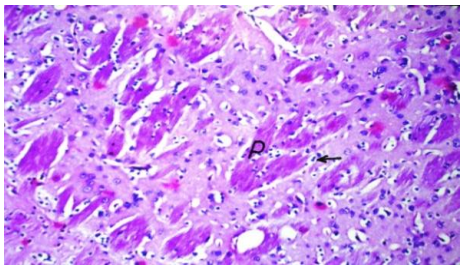

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Figure (9): Histopathological examination of rat brain of AD that received MSCs then NCD.

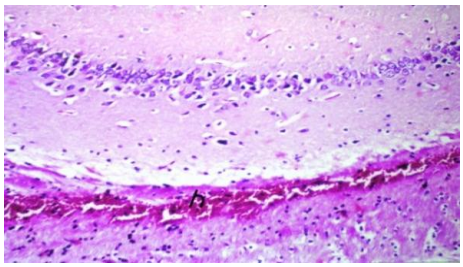

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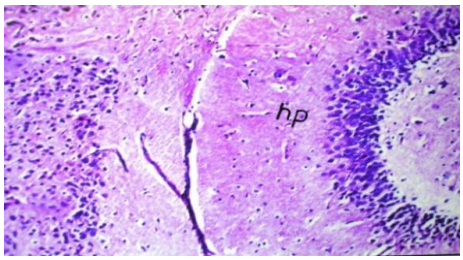
Figure (10): Histopathological examination examination of rat brain of AD that received MSCs.



H&E (X40)

Figure (11): Histopathological of rat brain of AD that received MSCs and NCD.

DISCUSSION

Alzheimer's disease (AD) is a neurodegenerative disease that leads to atrophy throughout the brain including the basal forebrain cholinergic system, amygdala, hippocampus and several other cortical areas. β-amyloid plaques and tau neurofibrillary tangles are hallmark features of AD **(15).** Current therapies are limited to provide only partial and temporary improvement of AD symptoms **(16).**

Stem cell therapy holds a great promise for the repair of injured tissues and organs, including the brain due to the unique ability to differentiate and self renewal**(2).** Curcumin may be a promising therapy for AD because it has different neuroprotective activities, including antioxidant, anti-inflammatory and antiamyloidogenic properties **(17).** One of the reasons suggested for the lack of beneficial results of curcumin in AD clinical trials has been the inability to produce sufficient brain levels following oral absorption **(18).** It has been repeatedly shown that curcumin has poor water solubility and poor oral bioavailability, and that much of an administered dose is excreted in the feces **(19, 5).** For this reason, we used in our study a novel curcumin derivative (NCD) to improve the bioavailability and delivery of curcumin.

In the present study, we aimed to evaluate the possible mechanisms of action of a novel curcumin derivative and MSCs in Alzheimer disease.

The pathophysiology of AD has not been clarified, but increasing evidence indicates that the loss of neurons in AD can be partly attributed to apoptosis**(20)**. Bax and Bcl-2 are

important apoptotic factors and are receiving increased attention from researchers [21, 22]. In the present study, the Bax gene expression was significantly increased and the Bcl_2 gene expression was significantly decreased in AD compared to the control group. Our results also coincide with the work of **Kudo et al.** [23] who injected oligomeric Aβ into the mouse hippocampus to induce neuronal cell death in 20 days. They reported that the number of neurons containing the active form of Bax was dramatically increased by Aβ injection, while the level of Bcl_2 was significantly decreased.

Curcumin is safe and can be used to antagonize apoptosis, tumor growth, oxidation, inflammation and hyperlipemia[24]. In our study, oral administration of NCD and pure curcumin significantly decreased BAX expression in AD. In addition, NCD significantly decreased BAX expression in AD compared to pure curcumin. On the other hand, NCD significantly increased Bcl_2 expression in AD while, pure curcumin increased Bcl_2 expression in AD but this difference was not significant suggesting that the NCD was more effective. Our study agrees with **Wang et al.** [25] who tested the effects of curcumin treatment on a rat model of Alzheimer's disease induced by beta-amlyoid (Aβ1-40).The results showed that Bax expression was remarkably decreased and Bcl-2 expression was increased in the rat Alzheimer's disease model after curcumin treatment. Curcumin can significantly improve spatial learning and memory functions in rats with Aβ1-40-induced Alzheimer's disease by modulating Bax and Bcl-2 expression.

Our results reported that the concomitant injection of MSCs and oral administration of NCD to AD rats provided a more significant decrease in BAX and increase in Bcl_2 expression compared to the AD +NCD group. Meanwhile, the injection of MSCs followed 2 weeks later by NCD showed a significant decrease in BAX but a non significant increase in Bcl_2.

In the present study, injection of MSCs to AD rats significantly decreased BAX gene and increased Bcl_2 gene expression.This agrees with the work of **Hai**[26] who injected MSCs into the tail vein after spinal cord injury in rat model to promote the restoration of motor function in the rat spinal cord injury repair. He showed that MSCs can do that in vivo by inhibiting the expression, regulation of apoptosis gene expression via increasing expression of Bcl -2, and Bax expression downturn.

In our study ,there was a significant increase in the mean SELADIN-1gene expression in the AD group receiving NCD, the AD group receiving MSCs then curcumin, the AD group receiving MSCs, and the AD group receiving MSCs+curcumin while in the AD group receiving pure curcumin, there was no significant difference as compared to AD group.Our results agree with the work of **Ahmed**[27] detected that brain seladin-1 and nestin genes expression were significantly decreased while, treatment with BM-MSCs produced a significant increase in their expression.This also agrees with **Abdel Aziz et al.** [3] who studied the effect of intravenously injected MSCs on

experimentally induced Alzheimer rats.They concluded that MSCs alone or with HO-1 induction exerted a therapeutic effect against the brain lesion in Alzheimer's disease possibly through decreasing the brain cholesterol level and increasing seladin-1 gene expression.

SiLu [28] found that curcumin can significantly inhibit the production of cultured in vitro neurons Aβ40/42, and the role is closely related to its activation of PPARγ and inhibition of BACE1. It was suggested that under stress conditions, reduced seladin-1 expression results in enhanced post-translational stabilization of BACE1 and increased β-amyloidogenic processing of APP. These mechanistic findings related to seladin-1 down-regulation are important in the context of AD as the oxidative stress-induced apoptosis plays a key role in the disease pathogenesis.

Antioxidant enzymes are important in preventing an excessive accumulation of ROS. Membrane lipids present in subcellular organelles are highly susceptible to free radical damage. Malondialdehyde (MDA) is a by-product of lipid peroxidation induced by free radicals and is also widely used as a biomarker of oxidative stress [29].

In the present study, there was a significant decrease in the mean GSH and a significant increase the MDA levels in the AD group compared to control group. **Gustaw-Rothenberget al.** [30] **and Pan et al.** [29] showed similar results.

Our results also showed a significant increase in the mean GSH and a significant decrease in the mean MDA levels in the AD group receiving the NCD , pure curcumin, MSCs then curcumin, receiving MSCs, and the AD group receiving MSCs+curcumin as compared to AD group. Our results agree with **El-denshary et al.**[31] who reported that administration of MSCs significantly decreased serum LDH, MDA and MPO enzymes and significantly increased GSH in the MSCs group in an experimental model of arthtritis compared to the control group.Also,when curcumin (5, 15, 45 mg/kg) was injected intraperitonealy (i.p.) once daily for a period of 10 days, it was able to decrease MDA and SOA levels significantly and improve learning and memory in rats of [32].

Curcumin has been demonstrated to have a strong antioxidant neuroprotective effects, scavenging ROS and neutralizing nitric-oxide-(NO-) based free radicals. The antioxidant activity of curcumin may be mediated through antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). Curcumin has been shown to serve as an acceptor, reacting with glutathione (GSH) and thioredoxin. Depletion in cellular GSH levels is an important measure of oxidative stress, which is implicated in the pathogenesis of AD. A study on postmortem brain of AD patients has revealed decreased levels of GSH in some area of the brain. It was demonstrated that curcumin is able to replenish the intracellular GSH pool. It was reported that curcumin mediates the direct detoxification of reactive nitrogen species such as peroxynitrite, thus exerting an antioxidant activity [18].

Our histopathological results agree with the work of **Ebrahimi et Al. [33]** who reported that upon transplantation into the brain, MSCs promote endogenous neuronal growth, decrease apoptosis, reduce levels of free radicals, encourage synaptic connection from damaged neurons and regulate inflammation. MSCs may encourage repair and new growth of neurons through providing neurotrophic factors (BDNF), which seems to be a significant aspect among the mechanisms of MSCs for the treatment of brain diseases.

Our results also agree with **Lee et al.[9]** who strongly suggested that intracerebral BM-MSC transplantation not only reduces amyloid load and tau phosphorylation in the brain, but also prevents cognitive decline and memory impairment, this occurs by activation of an endogenous microglial population with an alternative phenotype that has neuroprotective effects.

Our work is in accordance with recent advances in a study of AD performed by **Wang et al. [25]** who revealed that astrocytes (AS) are key factors in the early pathophysiological changes in AD. Glial fibrillary acidic protein (GFAP), a marker specific to AS, is markedly more manifest during morphological modifications and neural degeneration signature during the onset of AD. Several studies investigating the functionality of curcumin have shown that it not only inhibits amyloid sedimentation but also accelerates the disaggregation of amyloid plaque.

Conclusion: Administration of BM-derived MSCs either alone or with the NCD exerts a therapeutic effect on the brain lesion in Alzheimer's disease. This effect may be through the antioxidant and antiapoptotic action of both MSCs and curcumin.

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الطريقة المضادة لموت الخلايا المبرمج لعمل كلا من مشتق الكوركومين القابل للذوبان والخلايا الجذعية فى مرض الالزهايمر فى فئران التجارب

محمد طلعت عبد العزيز[١]، امين رزق[١] ، حنان حسني[١]، ليلي احمد راشد[١]، سهير محفوظ[٢] ، احمد طلعت عبد العزيز[١] و مروة القفاص[١]

١ - قسم الكيمياء الحيوية الطبية -وحدة الكيمياء الحيوية و البيولوجيا الجزئية - كلية الطب - جامعة القاهرة. ، ٢- قسم الباثولوجيا- كلية الطب - جامعة القاهرة.

الهدف : تهدف هذه الدراسة الى تقييم الطرق المحتملة التى يقوم بها كل من مشتق الكركومين والخلايا الجذعية فى علاج مرض الالزهيمر.قد اجريت هذه الدراسة على ٧ مجموعات تشمل كل مجموعة ١٠ فئران وهى كألاتى:المجموعة الأولى: الضابطة،المجموعة الثانية: المصابة بالالزهيمر،المجموعة الثالثة: المعالجة بـ ٨٠مجم من مستخلص الكركومين لمدة ٣ اسابيع،المجموعة الرابعة: المعالجة بالكركومين النهى لمدة ٣ اسابيع،المجموعة ،الخامسة: المعالجة بمشتق الكركومين ثم الخلايا الجذعية،المجموعة السادسة: المعالجة بالخلايا الجزعية،المجموعة السابعة: المعالجة بالخلايا الجذعية ومشتق الكركومين. وقد تم اجراء القياسات الاتية:التعبير الجينى لكل من (BCL2-BAX-SELADIN-1) ،مستوى الجلوتاثيون ومعامل الاكسدة بالتحليل الضوئى و دراسة المخ بالعرض المجهرى (الباثولوجى) . قد توصلت هذه الدراسة الى النتائج الاتية وجود الـ PLAQUE الخاصة بالمرض الالزهيمرفى الفئران المعرضة بمادة LPS مما يثيت اصابتها بالمرض وذلك بثبوت نتائج الباثولوجى بأنسجة المخ وقد اختفت هذه الـ PLAQUE اختفاء ملحوظا بعد العلاج خصوصا مشتق الكركومين مع الخلايا الجذعية بينما باقى المجموعات المعالجة كان هناك نقص فى الـ PLAQUE ولم تختفى كذلك كان هناك زيادة فى مستوى التعبير الجينى لكل من (BCL2-SELADIN-1) مصحوبا بنقص فى التعبير الجينى للـ BAX فى كل المجموعات المعالجة مع زيادة ملحوظة فى المجموعة العالجة للخلايا الجذعية والكركومين.مستوى (MDA) قد نقص بينما (GSH) قد ارتفع فى المجموعات المعالجة وخصوصا المجموعة المعالجة بالخلايا الجذعية والكركومين.مما سبق نستنتج أن استخدام الخلايا الجذعية بمفردها او مع مشتق الكركومين يمكن ان يكون لها تأثير علاجى ضد الاصابة بمرض اللالزهيمر وانه توجد طرق عديدة لعمل كل منهما يمكن ان تكون عن طريق مضادات الاكسدة ، أو نمو الخلايا الجذعية وتحولها لخلايا المخ.