Trimetazidine attenuates neuroinflammation *via* TLR4/NF-KB pathway inhibition in lipopolysaccharides-induced dementia in rats

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ABSTRACT

Neurodegenerative dementia impairs mental ability and short-term memory. This study investigated the effectiveness of trimetazidine (TMZ) in mitigating lipopolysaccharides-induced dementia in rats. Fifty male rats were randomly assigned into five groups; normal control, dementia, whereas other groups represented as dementia groups administered with 20, 40 mg/kg of TMZ, and 10 mg/kg of memantine, successively. Behavioral testing demonstrated a cognitive decline in dementia rats. Moreover, treatment with TMZ or memantine revealed a substantial decline in serum tau and phosphorylated tau with concurrent amelioration of serum Amyloid beta 1-42, neurogranin, 3-methoxy4-hydroxyphenyl glycol, APL1b25, APL1b27, and APL1b28 levels. Furthermore, such treatments yielded a significant down-expression of hippocampal toll-like receptor 4, NF-kB, cyclooxygenase-2, tumor necrosis factor-α, inducible nitric oxide synthase, caspase-3, and over-expression of Bcl-2 gene along with remarkable improvement in the hippocampal histoarchitecture as well as in the ionized calcium-binding adapter molecule 1 immunostaining compared the derangement in the dementia group. This study sheds light on TMZs neuroprotective effect on dementia pathophysiology by counteracting neuroinflammation. In conclusion, this study underscores the potential of TMZ in attenuating lipopolysaccharide-induced dementia in rats, offering significant cognitive and molecular benefits. The observed improvements in cognitive performance, serum markers, and hippocampal gene expression patterns collectively highlight TMZs neuroprotective capacity, suggesting its viability as a promising therapeutic intervention for addressing neurodegenerative dementia.

INTRODUCTION

Dementia is a progressive neurological illness that causes mental and behavioral decline, including, but not limited to, issues with communication, memory, reasoning ability, and other cognitive skills that impede day-to-day functioning. Such impairment in cognitive functions is commonly accompanied by deterioration in emotional control and social behavior. Vascular dementia, Alzheimer's disease (AD), and Lewy body dementia are all types of dementia [1,2].

About 50,000,000 people are thought to be affected by it at the moment, with that figure rising to an estimated 152,000,000 by 2050 [3]. As the aging rates universally increase, the rate of dementia incidence continues to grow, starting to make dementia a major issue for public health in low-income regions. The cost to society is high, and it is not only the quality of life diminished by dementia. In nations with a low per capita income, the charge is about $ 900, whereas in countries with a lower to moderate income, such as Egypt, the cost may reach $3,200 [4]. When it comes to diagnosing dementia, neuro-psychometric testing has been shown to be the most effective in low-income regions. Studies have shown that the biomarkers indicating dementia are Aβ40, Aβ42, total tau, and phosphorylated tau (P-Tau). These biomarkers are considered the most sensitive markers for diagnosing AD and cognitive impairment [4].

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Neuroinflammation is known to be implicated in the pathogenesis of dementia. It can be induced by systemic injection of lipopolysaccharides (LPSs), also known as endotoxins, which are complex molecules found in the outer membrane of Gram-negative bacteria, leading to the activation of pro-inflammatory signaling pathways *via* toll-like receptor 4 (TLR4), tau hyperphosphorylation, deposition of β-amyloid (Aβ) plaques, and ultimately cell apoptosis. LPS-induced neuroinflammation in the hippocampus is also associated with cognitive dysfunction, oxidative stress (OS), enhanced acetylcholinesterase activity, and activation of microglia and astrocytes [5]. Because of this, LPS has been used extensively in animal models to study the molecular underpinnings of cognitive impairment caused by neuroinflammation and to create a targeted therapeutic for alleviating neurological symptoms [6].

Up till now, there has been no curative therapy for dementia, and the most common currently used medications are acetylcholine esterase inhibitors that only delay the symptomatic progression and improve the cognitive and daily activities of patients with mild-to-moderate dementia. These drugs can be prescribed with memantine or used separately for moderate and severe cases [3]. Clinical studies of other therapeutic medicines targeting Aβ or P-tau as target pharmaceuticals have proven unsuccessful. As a result, novel therapeutic medication development is required [2].

Trimetazidine (TMZ), 1-(2,3,4-tri methoxy-benzyl) piperaciline hydrochloride, is an anti-ischemic agent [7]. Some studies proved that TMZ has anti-oxidative and anti-inflammatory properties [8]. This evidence illustrated that TMZ might be one of the appropriate candidates for counteracting neuroinflammation and prevention of cognitive decline following LPS injection. Therefore, this study was considered to elucidate the efficacy of TMZ in counteracting the pathological manifestations of dementia induced by LPSs through attenuating neuroinflammation and evaluating whether TMZ could differentially regulate the main biochemical and molecular markers in the pathological process of dementia.

MATERIAL AND METHODS

Materials

Chemicals and drugs

Pharmaceutical LPSs were purchased from Sigma-Aldrich Co., St Louis, MO; TMZ was obtained from Servier company, Egypt, whereas memantine was procured from COPAD Pharma Company, Cairo, Egypt. Any other chemicals used were locally purchased (Egypt) and were of high analytical grade.

Animals

Fifty male Sprague Dawley rats, adults weighing between 185 and 205 g, were maintained in the usual cages "under pathogen-free conditions and maintained under controlled room temperature and normal dark-light cycles. Animals were provided with standard chow and water *ad libitum*. Rats were adapted to these conditions for 2 weeks before the experimental protocol [9]."

In vivo study

The animals were arbitrarily categorized into five groups (10 rats/group): (1) *The negative control group,* which was intraperitoneally injected with a vehicle [0.5 ml of phosphate buffer saline (PBS)] four times/week for 2 weeks. (2) *The untreated dementia-induced group* was intraperitoneally injected with LPS (250 µg/kg) dissolved in PBS four times/ week for 2 weeks [10]. (3) *TMZ 20 group* denoted the dementia-triggered rats, which were administered per oral 20 mg/kg TMZ (dissolved in PBS) daily for 4 weeks, following the induction with LPS for 2 weeks [11]. (4) *TMZ 40 group* constituted the dementia-triggered rats treated orally with 40 mg/kg TMZ daily for 28 days [12]. (5) *Memantine group* comprised the dementia-triggered rats, which were administered orally with 10 mg/kg memantine (dissolved in PBS) as a reference drug daily for 4 weeks [13].

Methods

After animal treatment was over, the relevant behavioral assessments were performed, including the object recognition test (ORT), Y-maze, and Morris water maze (MWM).

Object recognition test

"Three days before testing, animals were subjected to 3 consecutive days of training in the dark open box, and each rat was also allowed to explore the apparatus without objects for 2 minute/day. On the testing day, a session of 2 minutes of two trials (T1 and T2) was performed. In the "sample" trial (T1), two identical objects were positioned in two opposite corners of the apparatus. The rat was located in the middle of the apparatus and was left to discover these two identical objects. After T1, the rat was placed back in its home cage, and an inter-trial interval of 1 hour was given. Subsequently, the "choice" trial (T2) was performed, where a new object replaced one of the objects that were presented in T1. Rats were exposed again to two different objects: the familiar (*F*) and the new one (*N*). To avoid the presence of a smell effect, the apparatus and the objects were carefully cleaned with alcohol solution after each trial. The objects' discovery was as follows; directing the nose toward the object at a distance of no more than 2 cm or touching the object with the nose. The times spent by rats exploring each object in T1 and T2 were documented manually using a stopwatch. Many variables were calculated as follows: a) The total time spent exploring the two identical objects in T1, b) the whole time spent exploring the two different objects in T2, c) the discrimination between F and N in T2 was measured by comparing the time spent discovering *F* with exploring *N*, and d) discrimination index (DI) represents the difference in exploration time expressed as a proportion of the total time spent exploring the 2 objects in T2. DI was calculated as follows: DI = N−F/N+F" [14,15].

Y-maze task

"The spatial memory function was evaluated by the Y maze task using the rewarded alternation. Each trial in this test comprised two runs. The first one was the sample run: during

which one of the goal arms was closed to force the rat to enter a particular arm, where it was rewarded with food. Before the consequent choice run, the second arm was opened, and the rat chose to enter either arm. If it entered the alternate arm, it received a food reward. Thus, correct performance depends mainly on memorizing which arm was entered in the sample run to guide proper response in choosing one. Each rat was subjected to five trials, with a 10-minute interval between them. The percentage of rewarded alternations was finally calculated" [16–18].

MWM test

MWM was carried out in a container (pool-like) about 180 cm in width, with a depth of 70 cm deep and an output faucet. The container was loaded with water to a depth of 35 cm with a white, non-poisonous, water-soluble stain. The temperature of the pool was retained at 26°C ± 1°C by the addition of warm water. A small, movable platform (about 9 cm in diameter) was placed in the middle of one of the pool's four imaginary quadrants. Before the acquisition phase or probing test, the rats were given 1 minute of free swimming.

"In the acquisition phase, rats were exposed to three training sessions daily, each of 60 seconds, for 4 successive days. During each trial, animals were left free to find the platform in the target quadrant. Once the rat positioned the platform, it was permitted to remain on it for 10 seconds. However, if an animal failed to reach the platform within 60 seconds, it was gently placed on the platform for 20 seconds. The escape latency was calculated as the average of the total time taken in all trials of each day of the acquisition phases to locate the platform. It was used as a measure of spatial learning. On the 5th day, a probe test (retrieval trial) was executed where the platform was removed, and each rat was placed in the water facing the pool wall starting from the quadrant opposite the platform quadrant and was allowed to explore the pool for 60 seconds. The time spent swimming in the target quadrant and the time spent in the non-target quadrant searching for the removed platform were considered the index of retrieval" [19].

Blood and tissue sampling

After performing the behavioral tests, after an overnight fast, the Sprague Dawley rats' blood was obtained, following receiving 10 mg/kg Midazolam (*via* IP route) for complete anesthesia, from the tail vein to separate the serum samples following spin at 400× g for 10 minutes at 5°C for biochemical analyses. After blood collection, the rats were sacrificed under complete anesthesia, and the brains were quickly dissected, thoroughly rinsed with ice-cold PBS , and blotted dry. The whole brain of a certain number of animals in each group was fixed in 10% neutral formalin buffer for 24 hours for histological and immunohistochemical examinations. While the whole brains of the rest of the animals in each group were dissected, hippocampi were extracted, immediately snap-frozen in cold nitrogen in the liquid state, and for the study of gene expression, kept at −80°C.

Biochemical determinations

Aβ1-42 residues (Aβ1-42), P-Tau, tau protein, and neurogranin serum levels were evaluated using ELISA kits bought from Wuhan Fine Biotech Co. LTD, Wuhan, China, according to the associated manufacturer's manuals. While APL25, APL27, APL28, and 3-methoxy4-hydroxyphenyl glycol (MHPG) were analyzed in the serum using ELISA kits supplied by SinoGeneClon Biotech Co., Hangzhou, China.

Molecular analyses

Total RNA was extracted from the hippocampi tissues of rats in each group using an RNeasy mini kit for total RNA purification from animal cells (Cat#74104, Qiagen, Hilden, Germany) according to the provided pamphlet. The integrity of the isolated RNA was assessed by a NanoDrop 2000 (Thermo "Fisher Scientific, Rockford, IL) using a 260/280 nm ratio. After that, RNA was reverse transcribed into cDNA using Revert Aid first strand cDNA synthesis kit (Thermo Fisher Scientific, Vilnius, Lithuania) according to the manufacturer's instructions" as per Mahmoud*et al.*, [20]. Then, the mRNA levels of TLR4, cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), Caspase-3, BCL-2, nuclear factor kappa B (NF-κB), and inducible nitric oxide synthase (iNOS) genes were assessed using QuantiTect SYBR Green polymerase chain reaction (PCR) Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. DNA-Technology Real-Time PCR device (DTlite 4, Moscow, Russia) was employed to perform the quantitative PCR cycles. "The thermal conditions were adjusted as follows; initial denaturation cycle at 94°C for 15 minutes, followed by 40 cycles of denaturation at 94°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds" as per Mahmoud *et al.*, [20]. Relative target mRNA quantification versus control was calculated using the comparative threshold cycle (Ct) method ($2^{-\Delta\Delta Ct}$). All values were normalized to the GAPDH gene. The primer pairs of the target genes are listed in Table 1.

Histological examination of brain tissue

After fixation of rat brain tissue samples in 10% formalin for 1 day, the specimen was treated sequentially with dilutions of diethyl alcohol and evaporated in xylene. A paraplast tissue embedding medium was used to infiltrate the cells, and subsequently, they were embedded. Five microns thick sagittal brain slices taken by the rotatory microtome were placed on glass slides to show off the many hippocampus structures in the specimens. Hematoxylin and eosin staining of tissue slices was performed as a standard procedure before microscopic analysis [29].

Immunohistochemical investigation

Immunohistochemical staining

In accordance with the manufacturer's guidelines, immunohistochemical staining for ionized calcium-binding adapter molecule 1 (IbA-1) was conducted. The secondary antibody Horseradish peroxidase (HRP) secondary antibodies Envision kit (DAKO, Hamburg, Germany) was incubated with the anti-IbA-1 antibody (ab108539—Abcam, Cambridge, USA—1:100) for 20 minutes after blocking the antigen-retrieved brain tissue slices with 3% hydrogen peroxide for 15 minutes.

Table 1. List of primer sequences of neuroinflammation-related genes used in qRT-PCR.

Genes	Primer sequences	References
TLR-4	F: 5'-TGGCATCATCTTCATTGTCC-3'	[21]
	R: 5'-CAGAGCATTGTCCTCCCACT-3'	
NF-κB	F:5'-GCACGGATGACAGAGGCGTGTATAAGG-3'	[22]
	R: 5'- GGCGGATGATCTCCTTCTCTCTGTCTG -3'	
TNF-α	F: 5'-CAGACCCTCACACTCAGATCATCTT-3'	[23]
	R: 5'-CAGAGCAATGACTCCAAAGTAGACCT-3'	
GAPDH	F: 5'-CACCCTGTTGCTGTAGCCATATTC-3'	[24]
	R: 5'-GACATCAAGAAGGTGGTGAAGCAG-3'	
COX-2	F: 5'-CTCCTTGAACACGGACTTGC-3'	[25]
	R: 5'-TCAGGGAGAAGCGTTTGC-3'	
Caspase-3	F: 5'-TGGTACCGATGTCGATGCAGC-3'	[26]
	R: 5'-GGTCCACAGGTCCGTTCGTT-3'	
Bcl-2	F: 5'-TGACTTCTCTCGTCGCTACC-3'	[27]
	R: 5'-CATCTCCCTGTTGACGCTCT-3'	
iNOS	F: 5'-TTGGAGCGAGTTGTGGATTGTT-3'	[28]
	R: 5'-TAGGTGAGGGCTTGCCTGAGTG-3'	

After that, "the tissue sections were PBS-washed and incubated with diaminobenzidine for 10 minutes. Counterstaining with hematoxylin was performed, followed by dehydration and clearing in xylene. Tissue sections were then cover-slipped for microscopic examination," as per Abbas *et al.* [30].

Quantitative immunohistochemical evaluation

Six far unplanned areas of the hippocampal CA3 subregions were scanned and analyzed to determine the mean positive counts of IbA-1/++ microglial cells in each immunostained tissue section with anti-IbA-1. "All morphological examinations, photographs as well as quantitative analyses were recorded using Leica Application system modules for histological analysis (Leica Microsystems GmbH, Wetzlar, Germany)" as per Ibrahim and Abdel Rasheed [29].

Statistical analysis

The current data are expressed as the mean ± SD. "Data were processed by one-way ANOVA followed by the Tukey *Post hoc* test except for the immunohistochemical scoring; statistical analysis was carried out by nonparametric Kruskal–Wallis *H*-test, followed by Dunn's test. $p < 0.05$ was considered to be statistically significant. GraphPad Prism software (version 9; GraphPad Software, Inc., San Diego, CA) was employed to perform the statistical analysis and to establish the graphical representation" [9,31].

RESULTS

Behavioral tests

Object recognition test

As depicted in Figure 1, dementia, TMZ (20 or 40 mg/kg), and memantine groups exhibited shorter T1 time (ORT) than the negative control group by 63%, 70%, 69%, and 82%, respectively. Dementia and memantine groups depicted significantly shorter T2 time (ORT) than the negative control group by almost 40% and 44%, respectively. Conversely, the treatment with TMZ (20 or 40 mg/kg) elongated the T2 time (ORT) compared to the untreated dementia group by 1.7 and 2-fold, respectively, with statistically comparable time to the negative control group. The ORT novel object time was reduced in dementia and memantine-treated rats by 82% and 51% versus the negative control group, respectively. This diminution was reversed by the treatment with TMZ (20 or 40 mg/kg) by six and seven-fold compared to the dementia group, respectively. The results were statistically comparable to the negative control group.

Y-maze task

Figure 2 represents the *Y*-maze test performed by all the experimental groups. The dementia-induced rats, as well as dementia rats treated with TMZ (20 mg/kg), experienced a decline in the percentage of rewarded alternations of rats in the *Y*-maze apparatus by 75% versus the healthy rats. On the other side, administration with TMZ (40 mg/kg) or memantine depicted a prominent elevation in rats' rewarded alternations by about 3.6 and 2.9-fold, respectively, versus the untreated dementia group, reaching the normal values.

Morris water maze

On the fourth and fifth training days, the dementia-induced rats portrayed significantly higher training time latency than the negative control group by approximately 1.7 and 2-fold, respectively. In the probe trial, treatment with either TMZ (20 or 40 mg/kg) or memantine decreased the exploration time in the target quadrant by 27%, 42% and 27% compared to the dementia group, respectively (Fig. 2). Noteworthy, the exploration time of rats treated with TMZ (40 mg/kg) reached the normal values.

Biochemical determinations

Figure 3 constitutes the influence of treatment with TMZ (20 or 40mg/kg) and memantine on the serum levels of dementia-related biomarkers in dementia-triggered rats. The positive dementia rats discovered a substantial rise in the serum tau and P-Tau levels along with a substantial decline in the serum levels of Aβ (Aβ1-42), neurogranin, APL1b25, APL1b27, APL1b28, and MHPG against the normal control rats. Whereas, administration of either TMZ (20 or 40 mg/kg) or memantine in the dementia-afflicted rats significantly decreased the serum levels of tau and P-Tau with a concurrent significant amelioration of the serum levels of Aβ (Aβ1-42), neurogranin, APL1b25, APL1b27, APL1b28, and MHPG ($p > 0.05$) [except for TMZ 20 group that showed a slight improvement in the serum APL1b28 level] in comparison with the untreated dementia rats. Worth mentioning, treatment with TMZ (40 mg/kg) significantly modulates the serum levels of Aβ (Aβ1-42), APL1b25, APL1b27, APL1b28, and MHPG when compared with TMZ 20 group. Moreover, the TMZ 40 group experienced a significant refinement in serum levels of APL1b28 and MHPG versus the memantine-
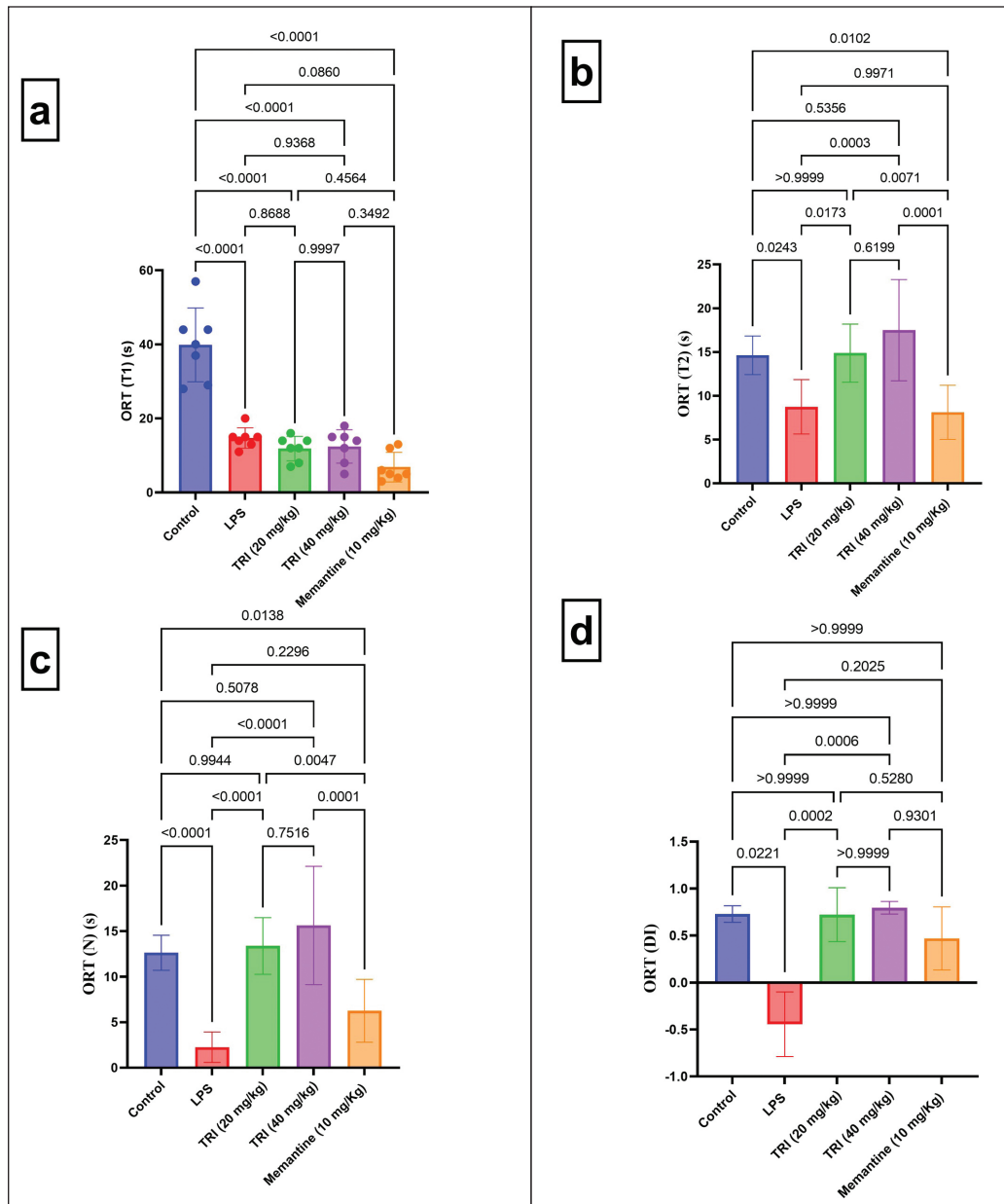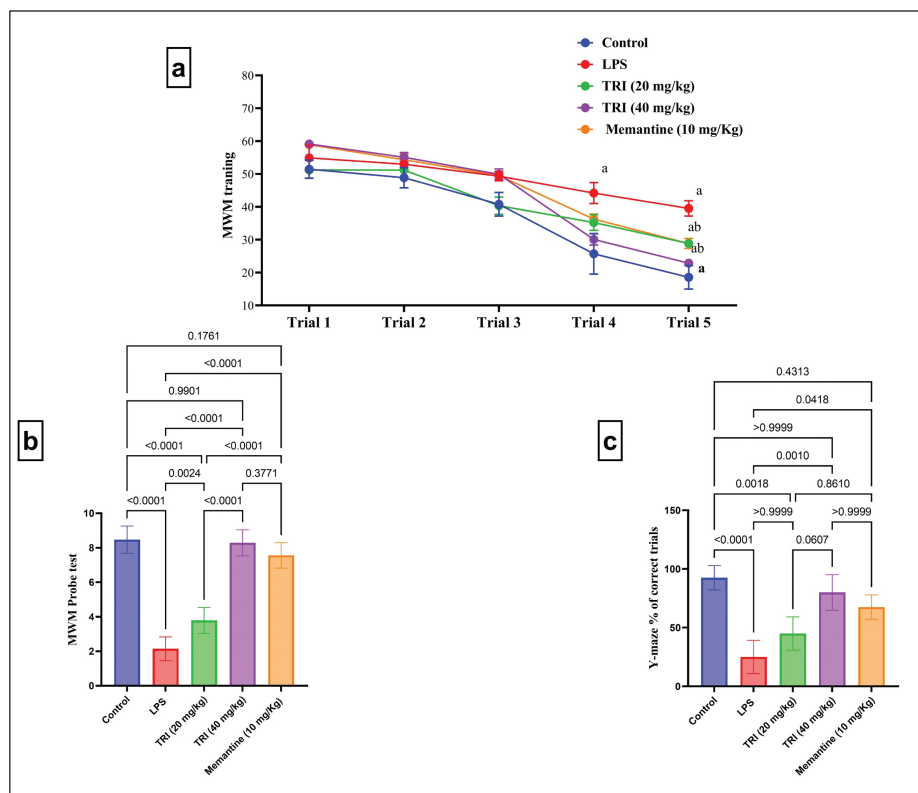
Figure 1. Effect of TMZ and memantine administration on ORT in different experimental groups. (a) ORT T1 with $F = 38.61$ (DFn = 4, DFd = 30, and $p < 0.0001$); (b) ORT T2 with $F = 9.932$ (DFn = 4, DFd = 30, and $p < 0.0001$); (c) ORT N with $F = 17.63$ (DFn = 4, DFd = 30, and $p < 0.0001$); and (d) ORT DI with $K = 23.36$ (colums = 5, total number = 30, and $p < 0.0001$). Data are presented as mean ± SD except for ORT DI, which is presented as median ± IQR.

treated group. Furthermore, the dementia rats treated with memantine significantly attenuated the serum levels of Aβ (Aβ1-42) and APL1b25 versus those treated with TMZ (20 mg/kg).

Molecular analyses

Figure 4 comprises the influence of TMZ and memantine administration on the transcriptional levels of neuroinflammation-related genes in the dementia-induced model. Dementia rats experienced a significant over-expression of TLR4, NF-κB, TNF-α, COX-2, iNOS, and caspase 3, accompanied by a significant down-expression of BCL-

2 compared to the negative control group. On the contrary, dementia-induced rats treated with either TMZ (20 or 40 mg/ kg) or memantine demonstrated a significant diminution in the gene activity patterns of TLR4, NF-κB, TNF-α, COX-2, iNOS, and caspase 3 accompanied by a significant upregulation of BCL-2 gene in comparison with the untreated dementia-induced rats. Notably, dementia rats treated with 40 mg/kg of TMZ revealed a significant down-expression of TLR4, iNOS, and COX-2, parallel with a significant elevation of the gene expression level of BCL-2 with respect to those treated with either 20 mg/kg TMZ or memantine.

Figure 2. Influence of TMZ and memantine administration on *Y*-maze and MWM tasks in different groups. (a) MWM training; (b) MWM probe test with $F = 105.5$ (DFn = 4, DFd = 30, and $p < 0.0001$); and (c) *Y*-maze % correct trial with $K = 31.65$ (colums = 5, total number = 30, and $p < 0.0001$). Data are presented as mean ± SD except for *Y*-maze, which are presented as median ± IQR. [a] Significance versus negative group and [b] Significance versus dementia group at $p < 0.05$.

Histological observations

Figure 5 illustrates the microscopic investigation of brain tissue samples (Hippocampal/CA3) of the different experimental groups. Brain tissue samples obtained from negative control rats showed normal organized histological features of hippocampal layers, including pyramidal neurons with intact nuclear details *(black arrow)* and intact intercellular brain matrix. Samples obtained in the dementia-induced group revealed significant neuronal injury and loss, with several instances of deteriorated or necrotic hypereosinophilic neurons losing their subcellular details with moderate intercellular edema *(blue arrow)* and minor records of apparent intact pyramidal neurons *(black arrow)*. Moderate higher records of reactive glial cell infiltrates were also shown *(arrowhead)*. On the contrary side, hippocampal sections of the TMZ 20 group demonstrated moderate neuroprotective efficacy with few records of degenerative neuronal changes *(blue arrow)* alternated with many figures of well-organized apparent intact neurons *(black arrow);* however; persistence of higher forms of glial cell infiltrates was observed *(arrowhead)*. Interestingly, the microscopic observation of hippocampal sections taken from the TMZ 40 group indicated a remarkable protective efficacy with minimal scattered records of degenerative neuronal changes *(blue arrow)* and apparent intact neurons *(black arrow)* with milder glial cell infiltrates *(arrowhead)*. The hippocampal samples of the memantine group displayed lower scattered records of degenerative neuronal changes *(blue arrow)* and intact neurons *(black arrow)* with milder glial cell infiltrates *(arrowhead)*.

Immunohistochemical findings

IbA-1 expression in the hippocampal tissue was determined by immunohistochemical technique to estimate the activation of microglia in response to inflammation triggered by LPS administration. Figure 6 illustrates the representative photomicrographs of IbA-1 immunostaining of hippocampal tissue of the dissimilar groups. Hippocampal sections attained from the negative control group showed a lack of IbA-1 immunostaining. In contrast, those obtained from the dementia group demonstrated significantly elevated expression of IbA-1 as compared to the negative control group, indicating the microglial activation following LPS supplementation. On the opposite side, the hippocampal sections of dementia-induced rats treated with 20 mg/kg TMZ revealed moderate IbA-1 immunoreactive cells. While those dementia rats received 40 mg/kg, TMZ displayed a substantial drop in the number of IbA-1 immunoreactive cells compared to dementia-induced rats. The hippocampal
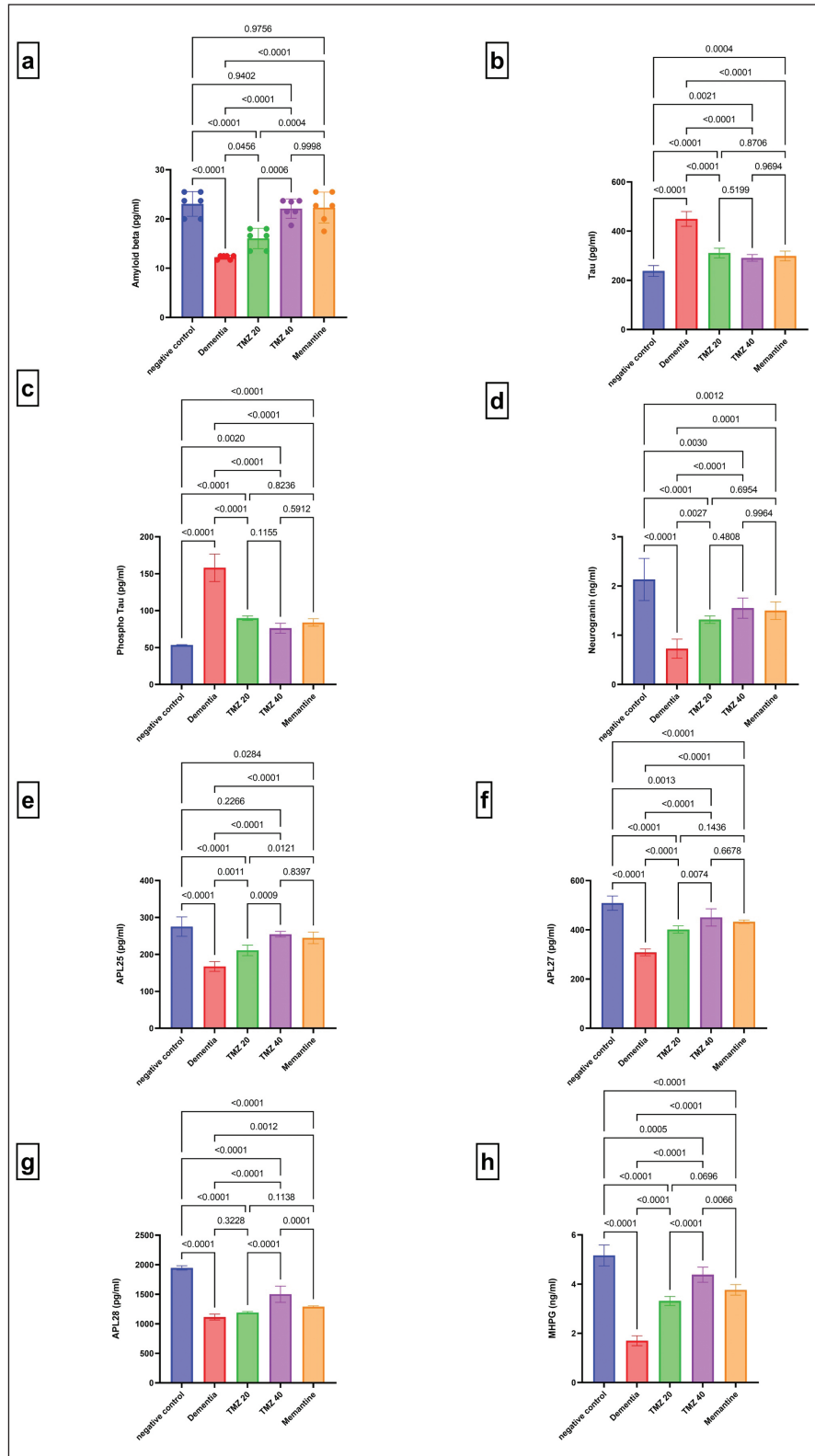
Figure 3. Influence of TMZ and memantine administration on serum levels of dementia-related biomarkers in dementia rats. (a) Aβ with $F = 28.16$ (DFn = 4, DFd = 25, and $p < 0.0001$); (b) Tau with $F = 79.38$ (DFn = 4, DFd = 25, and $p < 0.0001$); (c) P-Tau with $F = 108.5$ (DFn = 4, DFd = 25, and $p < 0.0001$); (d) neurogranin with $F = 25.53$ (DFn = 4, DFd = 25, and $p < 0.0001$); (e) APL25 with $F = 39.16$ (DFn = 4, DFd = 25, and $p < 0.0001$); (f) APL27 with $F = 64.01$ (DFn = 4, DFd = 25, and $p < 0.0001$); (g) APL28 with $F = 144.0$ (DFn = 4, DFd = 25, and $p < 0.0001$); and (h) MHPG with $F = 129.2$ (DFn = 4, DFd = 25, and $p < 0.0001$). Data are presented as mean ± SD.
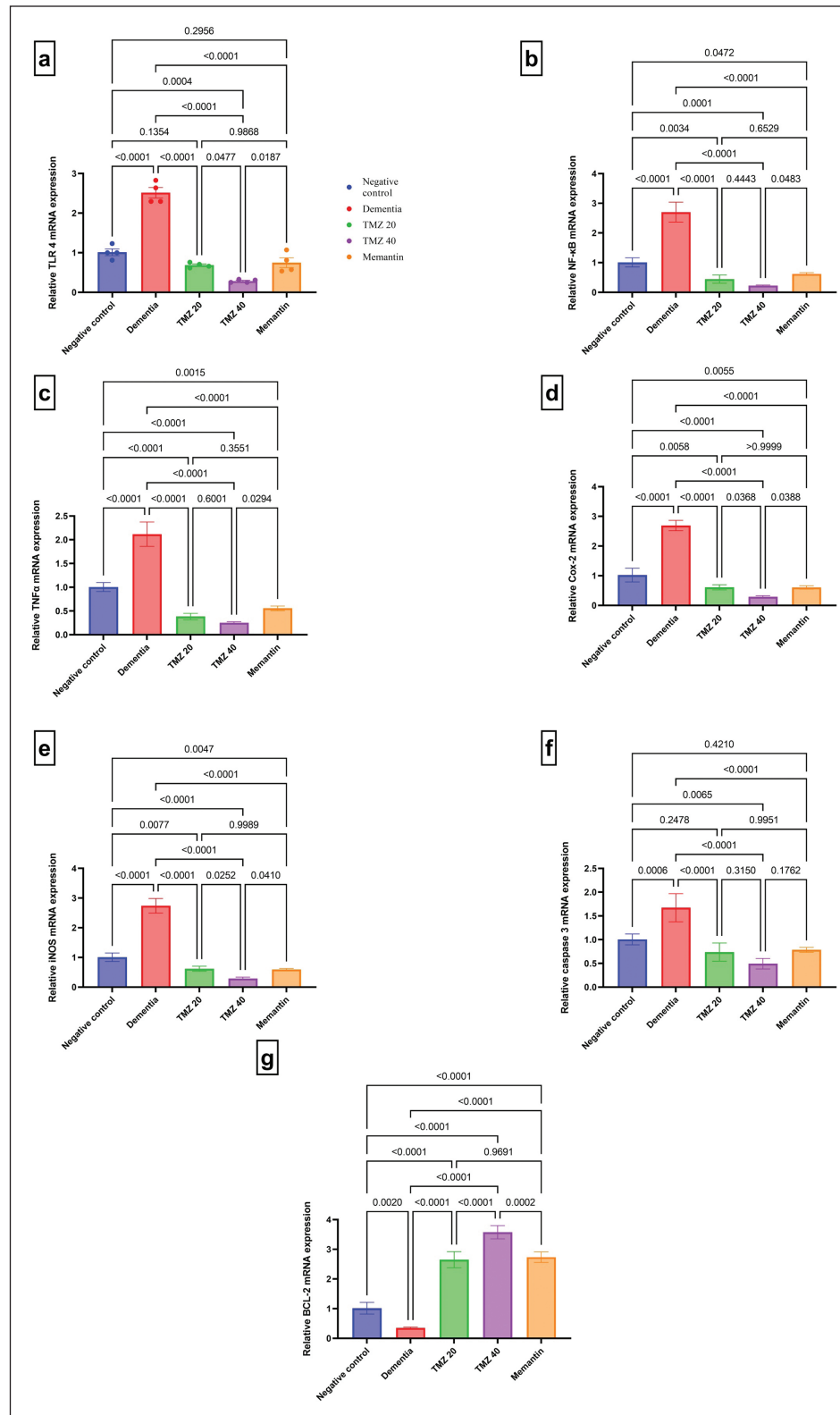
Figure 4. Impact of TMZ (20 or 40 mg/kg) and memantine administration on gene expression patterns of (a) TLR4 with $F = 89.35$ (DFn = 4, DFd = 15, and $p < 0.0001$); (b) NF-κB with $F = 124.6$ (DFn = 4, DFd = 15, and $p < 0.0001$); (c) TNF-α with $F = 139.4$ (DFn = 4, DFd = 15, and $p < 0.0001$); (d) COX-2 with $F = 189.7$ (DFn = 4, DFd = 15, and $p < 0.0001$); (e) iNOS with $F = 209.7$ (DFn = 4, DFd = 15, and $p < 0.0001$); (f) Caspase 3 with $F = 26.38$ (DFn = 4, DFd = 15, and $p < 0.0001$); and (g) BCL-2 with $F = 182.6$ (DFn = 4, DFd = 15, and $p < 0.0001$) in different groups. Data are presented as mean ± SD.
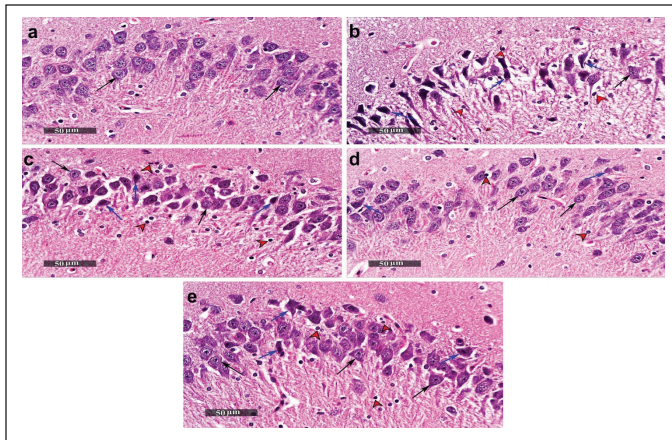
Figure 5. Histological examination of hippocampal tissue sections of the different groups; (a) negative control group; (b) dementia group; (c) TMZ 20 group; (d) TMZ 40 group; and (e) memantine group (Scale bars: 50 μm).
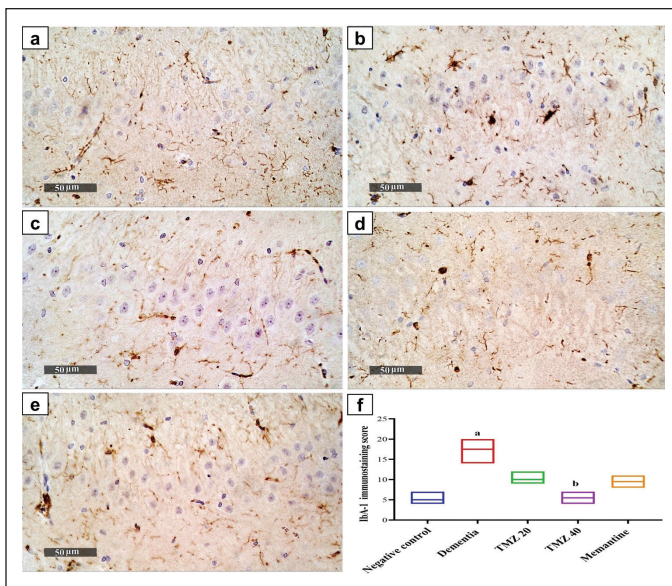
Figure 6. IbA-1 immunopositive cells in the hippocampal tissue sections of the different groups; (a) negative control group; (b) dementia group; (c) TMZ 20 group; (d) TMZ 40 group and (e) memantine group (scale bars: 50 μm), (f) IbA-1 immunostaining score with $K = 25.46$ (colums = 5, total number = 30, and $p < 0.0001$). Data are presented as median ± IQR ($n = 6$). [a] Significance versus the negative control group and [b] Significance versus dementia group at $p < 0.05$.

tissue sections of the dementia rats treated with memantine demonstrated low expression of IbA-1.

DISCUSSION

Neuroinflammation is the common hallmark of many neurodegenerative diseases, including AD, Parkinson's disease, and multiple sclerosis [32]. Abnormal aggregation of extracellular Aβ plaques in the brain is one of the pathological markers of AD. Aβ activates the inflammatory pathway and tau phosphorylation, resulting in neurotoxicity through free radicals and nitric oxide (NO) production. NO stimulates the secretion of excitatory glutamate, which ultimately triggers the "N-methyl-d-aspartate (NMDA) receptor," which ultimately leads to neuronal cell death [33].

According to our findings and in comparison with the negative controls, dementia rats experienced a decline in cognitive functions, as indicated by more extended periods in the learning acquisition phase, longer times on the MWM probing test with a lesser ORT discriminating index, and a lower number of correct trials in the Y-maze. These findings are greatly supported by those of Lee *et al.* [34] who indicated impairment in cognitive memory as elucidated by a remarkable upsurge in the time for familiar object exploration and a decline in the DI. This supposes a great brain degeneration after exposure to memory-impairing events, leading to a decline in episodic memory and recognition ability [35]. ORT evaluates the reactions of animals to unfamiliar and familiar objects [36]. Rats are subjected to ORT to examine their ability to recognize a novel object as their natural habit and stimulus recognition is subjected to evaluation [37]. Memory consolidation is hippocampus-dependent but not persistent. During the ORT, spatial or contextual object features are moved in the brain [38]. When a memory is retrieved in the context of novelty, it becomes labile and needs stability. Reconsolidation reorganizes previously established memories to add new knowledge [39]. Spatial memory consolidation is linked to hippocampal neurogenesis [40]. The MWM test was initially devised to assess the spatial learning and memory of mice [41]. This approach is reliable for evaluating cognitive function and visual short-term memory [42]. Similarly, the Y-maze task has been demonstrated to indicate hippocampal injury, evaluate cognitive deficits, and assess the impact of different medications on cognition [43].

The data in the current approach indicated that the dementia group exhibited a substantial drop in Aβ1-42 serum level. Recent research has focused on a blood-based Aβ screening tool for AD diagnosis since it is less intrusive, cost-effective, and widely available than cerebrospinal fluid (CSF) analysis and amyloid PET scans. Lower Aβ 1–42 serum levels enhanced the risk of AD as they were linked to the decreased hippocampus volume in the elderly, suggesting that when Aβ 1–42 deposits in the brain, plasma Aβ levels drop, leading to hippocampal atrophy [44]. It has also been mentioned that Aβ$_{42}$, as well as the Aβ42/Aβ40 ratio in plasma, were significantly lower in AD patients and inversely correlated with *in vivo* measures of fibrillar brain Aβ load measured by Pittsburgh Compound B [45]. Plasma Aβ levels have also been found to drop in those at risk for developing AD, and the low plasma levels of Aβ mirror the decline of Aβ in CSF to be indebted to amplified accumulation in the brain [46]. In addition, in the Tg2576 transgenic mouse model of AD, increased amyloid deposition in the brain was linked to concomitant decreases in A levels in CSF and plasma [47]. Sundelöf *et al.* [48] suggested that boosted accumulation of A in the CNS preceding the beginning of clinically evident cognitive impairment may account for the correlation between low plasma Aβ and the risk of AD.

In the current investigation, blood tau protein levels were significantly higher in the dementia group than in the negative control group. This outcome fits the study of Mattsson *et al.* [49] who found elevated plasma tau levels in

individuals with AD dementia. Moreover, research showed a strong correlation between plasma tau and cognitive decline, brain shrinkage, and hypometabolism in AD patients during follow-up. Tau, which usually resides in the brain's interstitial fluid, has been linked to extracellular leakage in AD. Plasma tau partly reflects brain pathology and high plasma tau reflects fast disease progression in the last stages. A recent study by Foiani *et al.* [50] indicated that plasma tau levels are raised in frontotemporal dementia, a neurodegenerative condition that causes personality changes or language difficulties.

The dementia group showed significantly higher serum P-Tau levels versus the normal control group. Serum P-Tau level is a portion of the brain's CSF-secreted P-Tau and indicates brain pathophysiological changes. Thus, it is considered a promising AD diagnostic marker [51]. AD patients have higher plasma P-Tau181 than controls. Such elevation begins in the early AD stage and increases throughout later stages, which coincides with CSF P-Tau181 elevation [52]. In individuals with Lewy body dementia, plasma P-Tau217 is related to plasma P-Tau181 and tau-positron emission tomography (PET) signal in the temporal brain. Plasma P-Tau may be a marker for AD co-pathology in Lewy body dementia [53].

The current research indicates that serum neurogranin showed significantly lower levels in the dementia group than in the negative control group. Neurogranin, a postsynaptic protein in dendrites, is released by excitatory neurons in the neocortex and hippocampus. It regulates synaptic plasticity, such as long-term potentiation and depression, which are crucial to memory consolidation [54]. AD patients were reported to have greater CSF neurogranin concentrations than cognitively healthy people [55], indicating synaptic degradation, which is associated with cognitive impairment [56]. Recent research by He *et al.* [57] found that neurogranin in neuron-derived exosomes (NDEs) in plasma showed the opposite pattern, possibly owing to transit from plasma to CSF. Furthermore, it has been found that NDEs in plasma declined in AD patients during the course of the illness and were even repressed in the years leading up to the beginning of the disease [58].

Serum APL25, APL27, and APL28 levels were significantly lower in the dementia group than in the negative control group. Amyloid precursor protein (APP) is a type I transmembrane protein. It is implicated in AD because of its proteolytic processing *via* the amyloidogenic pathway, which generates neurotoxic Aβ peptides that provoke AD's extracellular amyloid plaques [59]. APP belongs to a gene family including APLP1 and APLP2 [60]. It has been proposed that APLP1 also can be managed by γ- or β–secretase, such as APP, to produce simple Aβ-like peptides. These include some changes in amino acids "denoted APL1β25, APL1β27, and APL1β28, respectively" [61]. APLP1-based peptides may be a reliable alternative biomarker for Aβ 1-42 formation in the brain's tissue [62]. Thus, the decreased serum levels of the three APL1 peptides in the dementia group may be due to overexpression of APP and competition between APP and APLP1 for β- and γ-secretase processing, leading to an increase in Aβ1-42 deposition in the brain and a reduction in Aβ1-42 and APLP1-derived peptides in the serum, as shown in the present study.

The current attempt demonstrated a reduced serum level of MHPG in the dementia group. This result is consistent with Dekker *et al.* [63], who recorded a significant decrease in the MHPG serum level of dementia patients as compared to healthy subjects. German *et al.* [64] reported a correlation between the severity of the clinical manifestations of AD and noradrenergic deficits. Noradrenaline (NA), produced from dopamine (DA), is the precursor of MHPG and is considered the primary neurotransmitter of the sympathetic branch of the peripheral nervous system and brain. MHPG can freely cross the blood–brain barrier in contrast to NA, reflecting the main NA activity. Thus, plasma MHPG levels have been utilized to indicate the noradrenergic metabolism in the brain [65]. It has been reported that the decreased NA concentrations in brain areas and serum lead to diminished blood levels of the freely diffusing MHPG [63].

The current investigation demonstrated a significant elevation of hippocampal TLR4 and NF-κB gene expression levels in dementia-induced rats conforms to the study of Zarezadeh *et al.* [5]. In addition, the hippocampal tissue of dementia rats revealed significant overexpression of TNF- α, iNOS, and COX-2 genes, which are in agreement with the study of Yeo *et al.* [2]. Toll-like receptors are essential in recognizing invading pathogens and initiating immune responses. TLR4 exists as a complex with the co-receptor myeloid differentiation protein-2 (MD-2), expressed on the microglia surface. It has been suggested that LPS could induce dementia *via* triggering TLR4–MD-2 complex dimerization, resulting in the induction of the downstream NF-κB signaling pathway mediating the expression of pro-inflammatory mediators, including COX-2, TNF-α, IL-1, IL-6, and iNOS, which are responsible for neuroinflammation and neurodegeneration [66].

The present attempt demonstrated that the dementia group showed a significant up-regulation of the hippocampal caspase-3 gene accompanied by a substantial down-expression of the BCL-2 gene. These results are in harmony with those of Zhang *et al.* [67] and Khan *et al.* [68]. It has been reported that dementia induced by LPS is initiated by neuroinflammation, which stimulates the mitochondrial apoptotic pathway and apoptotic neurodegeneration. The process of causing neuronal cell death by LPS *via* the TLR4 pathway results in AP-1 activation, which further activates the JNK signaling pathway to inhibit BCL-2 proteins and activate the mitochondrial apoptotic pathway [69]. The activation of the mitochondrial apoptotic pathway upon LPS injection is triggered by Bax/Bcl-2 signaling. Activated Bax/Bcl-2 provokes the activation and release of cytochrome c, which in turn stimulates the activation of caspase cascades, including caspase-3, which is known to play an essential role in apoptotic neurodegeneration. The activated caspase-3 induces neural cell apoptosis and cleaves poly(ADP-ribose) polymerase 1, which causes damage to the DNA of neurons [68,69].

Zarifkar *et al.* [70] reported that intraperitoneal injection of LPS provokes neuroinflammation, hippocampal apoptosis, and beta-amyloid plaque formation in the hippocampus, leading to cognitive impairment and learning deficits. This explains the neuronal cell death and the pathological alterations observed in the dementia-induced group upon the histological investigation

of their hippocampal sections, which agrees with the result reported by Amraie *et al.* [71].

IbA-1 is a specific marker for microglia; the current study revealed a significant increase in the number of IbA-1-positive cells in dementia-induced rats. With this result, we agree with Ifuku *et al.* [72] who demonstrated a significant elevation in IbA-1-positive microglia in the hippocampus of LPS-injected rats. Microglia, the major brain macrophages, are known to play an essential role in the development of neuroinflammation, and microglial stimulation mainly contributes to central nervous system-related injuries. Under normal conditions, microglia phagocytize brain metabolic products, synaptic elements, and dead cells in the brain. Thus, once activated, microglia are recruited to the site of the lesion and eliminate cellular debris. Although microglial activation seems to be essential for host defense, persistent microglial activation can cause apoptosis of neural cells and the release of pro-inflammatory cytokines in the hippocampus. Aβ stimulates microglia and activates their production of pro-inflammatory mediators, such as TNF-α, NO, and interleukin (IL)-1β [32]. In LPS-induced dementia, the induction of macrophages and microglial cell activation occurs *via* stimulating the TLR4 signaling cascade, which in turn initiates inflammation as a result of the production of inflammatory mediators such as oxygen-free radical, NO, and pro-inflammatory cytokines, including TNF-α and IL-1β [5].

TMZ administration resulted in reduced cognitive impairment, which was demonstrated by shorter times in the learning acquisition phase and probing test of MWM with improvement in the DI of ORT accompanied by an increased number of correct trials in *Y*-maze. These results are in accordance with those of the study of Mohamed *et al.* [19] who declared the improvement of cognitive function in diabetic epileptic rats in response to TMZ intake. These investigators documented that TMZ reduced apoptotic markers and inflammatory cytokines and alleviated hippocampus neuronal damage-induced cognitive impairment. TMZ inhibits LPS-induced brain inflammation *by activating* antioxidant enzymes, promoting axonal regeneration and myelination, and preventing drug-induced hippocampal oxidative damage and experimental brain shrinkage [73]. In addition, TMZ enhances brain activity by protecting neuronal cells from intracellular acidosis, preserving the brain mitochondrial membrane, inhibiting lipid peroxidation, and regulating Na/Ca channels [74].

Neuronal excitement increases brain energy consumption from glucose metabolism, producing acetyl-coenzyme A, which synthesizes brain acetylcholine [75]. The cholinomimetic drugs reduced Aβ formation, which explains TMZs actions [76]. In addition, they slowed the AD progression and improved learning and memory functions in the Tg2576 AD mice model that overexpressed human APP [77].

Furthermore, the treatment of dementia groups with TMZ brought about a significant amelioration in the serum biochemical markers of dementia represented by serum Aβ, tau protein, P-tau, and neurogranin levels in addition to APL1β25, APL1β27, APL1β28, and MHPG serum levels. TMZ has been reported to restore energy metabolism in mitochondria [78], regulating the apoptosis rate in brain injury [79]. Moreover, TMZ has been found to be anti-inflammatory and block cytokine release

to restore blood–brain barrier integrity [80]. The capacity of TMZ to easily pass the blood–brain barrier and disperse across significant brain regions to impact numerous processes of brain damage is a crucial factor in the drug's neuroprotective effect [81]. Earlier studies demonstrated that TMZ possesses anti-oxidative and anti-inflammatory properties and plays cytoprotective roles in various tissues, including nervous tissue [82]. A more recent study reported that the antioxidant enzymes superoxide dismutase and catalase were both stimulated by TMZ, and malondialdehyde levels were lowered [8]. Moreover, Hassanzadeh *et al.* [73] reported that TMZ could increase seladin-1 mRNA levels in the hippocampus of the rat model of sporadic AD. Seladin-1 has been demonstrated to be resistant to β-amyloid deposition-induced toxicity and OS [83]. All of this evidence could explain the almost normal level of serum Aβ 1-42 and the reduced level of tau protein and P-Tau in the serum of the dementia group upon treatment with TMZ, as shown in the current study.

The pathogenesis of AD involves Aβ deposition that is responsible for stimulating synaptic impairment and neurodegeneration. The susceptibility of the brain to OS is deliberated to be a critical damaging issue in AD. Aβ and OS are closely related to one another since Aβ stimulates OS and the contrary. Evidence demonstrates that prolonged oxidative damage leads to the manifestation of AD symptoms, comprising Aβ buildup, formation of the neurofibrillary tangle, metabolic disorder, and cognitive dysfunction [84]. The significant recovery of neurogranin serum level to almost normal level in the dementia group treated with TMZ could be able to be accounted for TMZ's ability to reduce lipid peroxidation by tempering β-oxidation via hindering of β-keto thiolase (3-KAT) coenzyme and ensuring glucose uptake in the brain [78]. This antioxidant activity contributes to the neuroprotective action exerted by TMZ in neurodegenerative diseases triggered by pro-inflammatory mediators *via* lipid peroxidation [85]. This neuroprotective activity of TMZ is endorsed due to its potential to reduce fatal oxidation of mitochondria, motivate p-ERK 1/2 and p-AMPK signaling cascades, and reduce adenosine triphosphate-dependent energy disruptions. This was all displayed as a decline in neuronal death and conserved neuronal integrity [86]. Interestingly, mitochondria of rat brains have been found to contain binding sites for TMZ [87]. Previous *in vitro* investigations indicated that TMZ could hinder mitochondrial permeability transition pores' opening [88] and, thus, prevent apoptotic neuronal loss following brain injury. Therefore, one can postulate that the antioxidant, anti-inflammatory, and anti-apoptotic properties of TMZ enable it to reduce synaptic degeneration and restore neurogranin serum levels.

Treatment of the dementia groups with TMZ yielded a substantial improvement in the blood levels of APL25, APL27, and APL28, as well as MHPG. It has been reported that there is an equilibrium between Aβ formation and destruction *via* self-regulatory pathways [89]. However, the impairment of this balance leads to Aβ accumulation in the brain [90]. Essential pathways that interfere with this equilibrium are free radical injury and neuron inflammation [91]. Thus, the overproduction of reactive oxygen species (ROS) is considered a crucial factor of synaptic dysfunction and ultimately provokes senile plaque formation [92].

Furthermore, *in vitro* study by Muche *et al*. [93] indicated that oxidative damage gradually motivated APP expression levels in endothelial cells originating from cerebral cortical tissue of transgenic Tg2576 AD mice. In addition, oxidizing agents have been reported to surge the translation of APP [94] and escalate secreted Aβ levels [95]. Many antioxidants have been employed to decrease AD-related OS and defend cells against Aβ-dependent neurotoxicity [96].

Regarding the role of neuroinflammation in dementia, the "family pyrin domain containing 3" (NLRP3), an inflammasome, is a new cytosolic-multi-protein implicated with neuron inflammation and the body's natural defenses [97]. Thus, Aβ deposition in a transgenic AD mice model may be reduced by blocking the NLRP3 [98–100]. In addition, inflammatory mediators promote APP expression and the development of Aβ [101]. Anti-inflammatory medications may, thus, prevent or cure AD by preventing neuroinflammation, which reduces the generation of anti-inflammatory medications may thus prevent or cure AD by preventing neuroinflammation, which reduces the generation of Aβ [102,103]. Hence, the underlying mechanism by which TMZ could ameliorate the serum levels of APL25, APL27, APL28, and MHPG in the dementia group relies on the antioxidant and anti-inflammatory potential of this drug, which reduces the overproduction of APP and Aβ deposition in the brain with consequent protection of central neurons including noradrenergic neurons.

In the current study, TMZ administration produced a significant down-regulation of hippocampal TNF-α TLR-4 and NF-κB genes. These data are greatly supported by the findings of Su *et al*. [104] which demonstrated the decline of inflammatory markers, such as NF-κB and TNF-α, following TMZ treatment of coronary artery micro-embolism pig model. Furthermore, Ozturk *et al*. [105] commented that TMZ ameliorated the ovary ischemia-reperfusion injury *via* down-regulating the expression of TLR-4 and NF-κB proteins, attenuating OS and inflammation. TMZ has been reported to possess anti-inflammatory effects owing to its potential to have a major impact on lowering the concentrations of inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, in the bloodstream [80]. Moreover, TMZ reduces the release of pro-inflammatory mediators from macrophages induced by ROS, including C-reactive protein, TNF-α, IL-1, and interleukin 8 during both inflammation and ischemia [106].

In the present investigation, the hippocampal tissue of TMZ-treated rats illustrated a significant down expression of iNOS and COX-2 genes, which comes in line with the study of Natarajan *et al*. [107] who reported the decrease in the intestinal expression of iNOS, COX-2 in methotrexate-induced injury following TMZ treatment. Moreover, our findings revealed that TMZ treatment had reversed the mitochondrial apoptotic pathway and neurodegeneration by up-regulating BCL-2 transcribing genes and reducing the activated caspase-3 transcriptional activity in the hippocampal region of the dementia-induced rat brain. These findings are consistent with those of the study of Zhang *et al*. [108] which indicated that TMZ attenuated cardiac damage by hindering programmed cell death through decreasing Bax/Bcl-2 proportion and deactivating caspase-3 expression, sliced PARP, and reducing cytochrome c levels in the rats' myocardium. In addition, TMZ inhibited

oxidation strain and cardiac programmed cell death *via* activating Nrf2/HO-1 and suppressing NF-κB signal transduction. These outcomes could be attributed to the ability of TMZ to decrease the TLR4 expression, which in turn down-regulates the downstream signaling of NF-κB, resulting in inhibition of the pro-inflammatory mediators, including COX-2, TNF-α, IL-1, IL-6, and iNOS, which are the culprit for neuroinflammation and neurodegeneration. This is because dementia induced by LPS is commenced through neuroinflammation, causing apoptotic neurodegeneration *via* the TLR4 signaling cascade. Such signaling activates AP-1, stimulating JNK to interfere with BCL-2 and activate mitochondrial apoptosis [69]. Therefore, the mechanism underlying the over-expression of BCL-2 following TMZ treatment in the dementia group could be ascribed to the down-regulation of TLR4 and its downstream signaling pathway.

Histological examination of hippocampal sections obtained from the TMZ 40 group displayed little neuronal degenerative changes and intact neurons, indicating its notable neuroprotective efficacy. This discovery is as per the observations of Hassanzadeh *et al*. [73] who revealed the neuroprotective effect of TMZ against sporadic AD as confirmed by the attenuation of hippocampal CA3 neuron loss upon histological examination. Moreover, these investigators indicated that this beneficial effect of TMZ could be attributed to the free-radical scavenging activity and restoration of intracellular antioxidant activities. In our study, TMZ treatment displayed such a protective effect against neural damage, which may be due to its scavenging ability of ROS released upon LPS administration, resulting in ameliorating the OS and the inflammatory response induced by LPS.

In the present approach, TMZ administration resulted in the inhibition of the microglial activation, as validated by the substantial drop in IbA-1 immunopositive cells in the hippocampal regions of rat brains. This could be explained by the anti-inflammatory effect of TMZ responsible for hindering the TLR-4/NF-κB pathway [105], resulting in the deactivation of microglia and hence suppressing the inflammatory response.

Memantine is regarded as a neuroprotective agent for treating several dementias [109]. Memantine has been known as the antagonist of the NMDA receptor since it exerts its neuroprotective action against AD by blocking the N-methyl-D-aspartate receptor channel and hence alleviating glutamate-mediated neurotoxicity [33].

The present study indicated that dementia rats treated with memantine experienced a remarkable enhancement in novel object recognition time, DI in ORT, decreased training time latency on the fourth and fifth training days of the MWM, and a more extended exploration time in the probing test. Moreover, memantine treatment in dementia rats showed an improved alternating memory as indicated by the higher number of correct trials of *Y*-maze. Higaki *et al*. [42] reported that memantine treatment reduced age-related memory loss in LPS-induced neuroinflammation in rats.

The current approach demonstrated that dementia groups treated with memantine exhibited a significant recovery of the serum levels of Aβ, neurogranin, APL1β25, APL1β27, APL1β28, and MHPG together with a significant decrease in tau protein and P-tau serum levels. It has been stated that

the supplementation of rat cortical neuronal cultures with memantine reduced the secretion of Aβ1–42. Moreover, APP/presenilin-1 (PS1) transgenic mice treated with memantine exhibited significantly reduced cortical levels of soluble Aβ 1–42. Thus, memantine could reduce amyloid plaque formation and might inhibit the accumulation of fibrillogenic Aβ in mammalian brains [110]. Memantine is a cationic amphiphilic drug with a similar structure to lysosomotropic drugs that are known to accumulate in acidic cellular compartments and inhibit phospholipases [111]. The possible mechanism by which memantine could reduce the secretion of APP and Aβ peptides into the conditioned media of cell cultures could be attributed to the accumulation of memantine in lysosomes. Moreover, it has been suggested that memantine could alter the activity of the γ-secretase complex so that the majority of the Aβ produced is the shorter, less amyloidogenic Aβ1–40 [110]. All of this evidence supports the reason behind the recovery of Aβ1–42 serum levels in memantine-treated groups.

Memantine can repress the tau neuronal forma via the cap-independent triggered pathway. This means that memantine can be used as a potential tau internal ribosome entry site (IRES)-reliant on inhibition of translation as it inhibits the ability of tau-IRES, resulting in decreased tau expression. As a result, memantine's effect on AD might be attributable to its ability to inhibit both NMDA receptor-induced excitotoxicity and IRES-mediated translation [112]. The impairment of phosphoseryl/phosphothreonyl protein phosphatase (PP)-2A, known to regulate tau phosphorylation in the AD brain, is believed to be the causative factor of abnormal tau accumulation and hyper-phosphorylation. It has been reported that the NMDA receptor forms a complex with PP-2A. However, NMDA receptor overactivation in chronic neurodegenerative conditions leads to PP-2A dissociation from that complex, resulting in the reduction of PP-2A activity. It has been suggested that memantine could suppress the abnormal tau hyperphosphorylation and neurofibrillary degeneration in AD *via* modulating PP-2A signaling in addition to its effect as an NMDA receptor antagonist [113].

AD is characterized by several neuropathological hallmarks, including amyloid plaque deposition, loss of acetylcholine in the hippocampus, and generalized synaptic loss [114]. It has been reported the neuroprotective effect of memantine on neuronal cell culture models and in a transgenic AD mouse model is manifested by improved neuronal morphology and synaptic transmission in degenerating primary neurons, in addition to preserving synaptic markers. Memantine was shown to alter APP processing in AD animal models. APP and Aβ are associated with neurite and synapse regulation. In addition to lowering synaptic noise, memantine has been shown in preclinical studies to restore the normal physiological function of NMDA receptors. This means memantine restores impaired synaptic plasticity [115]. It is considered a neuroprotective agent by reducing glutamate-dependent toxicity and was found to boost the levels of "brain-derived neurotrophic factor," hence affecting rodent neuroplasticity [116]. Thus, memantine possesses the ability to recover synaptic plasticity and support the treatment of diseases interfering with cognitive function [117]. The

potency of memantine to stimulate synaptic transmission in the hippocampus is in harmony with the described positive impact on cognition dysfunction in humans [118]. These findings indicate that memantine enhances neurogranin through its potent effect on the restoration of synaptic impairment.

Regarding the effect of treatment of the dementia groups with memantine on APL25, APL27, and APL28 serum levels, it has been established that memantine administration reduced the brain levels of soluble and insoluble Aβ peptides inTg2576 mice, suggesting that memantine acts *via* reducing Aβ production through the regulation of intracellular APP trafficking [119]. Memantine treatment in the 5XFAD model of AD significantly reduced amyloid plaque pathology [120]. In addition, the study of Liu *et al*. [121] observed that the over-expressions of Aβ1-42 and APP were decreased in the brains of APP/PS1 mice upon treatment with memantine. A combination of mechanisms for this action of memantine may bypass the NMDA receptor and act on γ-secretase [110].

Concerning the effect of memantine on MHPG, it has been reported that away from its major action at the NMDA receptors, memantine may affect extracellular targets in the brain, e.g., affect dopaminergic and noradrenergic transmission or stimulate the expression of neurotrophic factors [122]. Treatment with memantine has been found to significantly increase the extracellular DA, NA, and their metabolites in the cortical regions [123]. Nerve growth factor (NGF) is a neurotrophin known to stimulate neuron differentiation, survival, and plasticity. Its deficiency in the brain induces neuron dysfunction and apoptosis, as well as accelerating Aβ deposits and Aβ-induced toxicity [124]. Memantine elicited beneficial neuroprotective effects on amyloidosis and cognitive improvement, possibly by increasing the endogenous NGF [121]. Scott *et al*. [125] suggested that NGF may be responsible for the regenerative growth of noradrenergic neurons following axonal damage in rat brains. Moreover, NGF signaling is implicated in elevating NA content and noradrenergic neuron-specific enzymes [126]. One can postulate that memantine could normalize the serum level of MHPG *via* regulating NGF signaling.

The current findings indicated that memantine treatment abrogated the neuroinflammation in dementia, as shown by the significant down-regulation of TNF-α, COX-2, iNOS, TLR-4, and NF-κB genes. These results are in line with those of Alomar *et al*. [127] who showed that memantine alleviated diabetic neuropathic pain *via* downregulating the TLR-4/NF-κB inflammatory axis, resulting in downregulating the pro-inflammatory cytokines such as TNF-α.

The present study also showed that dementia rats treated with memantine displayed down-regulation of caspase 3 with concurrent up-regulation of the BCL-2 gene. These findings are supported by Song *et al*. [33] who indicated that the AD cell model treated with memantine revealed a significant down-regulation of caspase-3 protein expression and over-expression of BCL-2, suggesting its anti-apoptotic activity against neuronal cell death.

Furthermore, the current data indicated a remarkable improvement in the pathological aberrations of hippocampal tissue of dementia rats treated with memantine. This observation conforms with that of Eldeeb *et al*. [128]. Such favorable

neuroprotective effect of memantine against dementia may be attributed to its anti-inflammatory and anti-apoptotic activity through NMDA receptor modulation, inhibiting the microglial activation and hence blocking the inflammatory cascade activation [129–131].

The current study revealed that memantine treatment resulted in mild IbA-1 immunostaining in the hippocampal tissue of dementia rats. This result fits Mei *et al*. [132], who revealed that memantine treatment attenuated microglial activation after repetitive mild traumatic brain injury in mice. This was validated by the significant decrease of IbA-1-positive cells in the hippocampus. Moreover, an earlier study by Rosi *et al.* [133] proved that treatment with memantine dramatically decreased the number of activated microglia during chronic neuroinflammation without affecting the number of resident microglia.

CONCLUSION

This study provides evidence for the neuroprotective effects of TMZ against lipopolysaccharide-induced dementia in a rat model, likely through inhibiting neuroinflammation via the TLR4/NF-κB pathway. TMZ treatment attenuated the behavioral and cognitive deficits, improved hippocampal pathology, modulated biochemical markers, and beneficially altered gene expression patterns related to dementia pathophysiology. These preclinical findings reveal the potential of TMZ as a promising therapeutic agent against neurodegenerative dementia. Further clinical studies are warranted to translate these results to humans and investigate the efficacy of TMZ for mitigating mitochondrial damage and neuroinflammation in patients with dementia.

The preclinical evidence from this study raises important implications for potential clinical applications of TMZ to treat neuroinflammation and mitochondrial dysfunction associated with dementia in humans. As a clinically approved drug for angina treatment, TMZ has a known safety profile in humans. The dosage used in this rat study (20–40 mg/kg) would translate to a human equivalent dose of 1.6–3.2 mg/kg based on body surface area conversion between species. This is within the clinical dosage range for TMZ. Therefore, repurposing TMZ as an adjuvant therapeutic in dementia patients could be a promising translational approach.

Clinical trials are needed to evaluate the efficacy of TMZ for modulating biomarkers, cognitive outcomes, and disease progression in patients with mild cognitive impairment and early AD. TMZ could be tested alone or in combination with standard dementia medications like cholinesterase inhibitors. Studies are also warranted to examine if TMZ may have a preventive effect to reduce dementia risk in susceptible populations. In addition, research should explore optimal dosing, safety, and pharmacokinetics of chronic TMZ administration in older adults.

Overall, this study provides a strong rationale for further investigation of TMZ in human clinical trials to assess its potential as a therapeutic agent that targets neuroinflammation and mitochondrial dysfunction relevant to dementia pathophysiology. The findings open promising avenues for developing effective adjuvant treatment strategies for neurodegenerative dementias.

AUTHORS' CONTRIBUTIONS

Nadia S Mahmoud: Conceptualization, molecular analysis, statistical analysis, writing—original draft, data visualization. Marawan A. Elbaset: Conceptualization, animal experiments, and behavioral assessment, statistical analysis, writing—review and editing, data visualization. Ola A.M. Mohawed: molecular analysis, statistical analysis, writing—original draft. Hanaa H Ahmed: Conceptualization, biochemical assessment, data validation, writing—review and editing. All authors wrote, revised, and approved the final manuscript.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study was conducted according to the guidelines for laboratory animal care and use and was approved by the "Ethical Committee of the Medical Researches of the National Research Centre, Egypt" (Approval No. 3415062021), complying with the "ARRIVE guidelines and was performed according to the National Institutes of Health Guide for the Care and Use of Experimental Animals (NIH Publications No. 8023, revised 1978)."

DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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