



Original Article

Asian Pacific Journal of Tropical Biomedicine



apjtb.org

doi: 10.4103/apjtb.apjtb_937_23

Neuroprotective effect of liraglutide and memantine in a rat model of Alzheimer's disease

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ABSTRACT

Objective: To assess the effect of memantine combined with liraglutide on aluminum chloride (AlCl₃) and *D*-galactose (*D*-GAL)-induced neurotoxicity in rats.

Methods: Male Wistar rats were divided into 5 groups of 5 animals each: the positive control, the negative control, the memantine-treated group, the liraglutide-treated group, and the combination group treated with memantine and liraglutide. AlCl₃ and *D*-GAL were used to induce neurotoxicity. Behavioral tests, brain beta-amyloid protein, and oxidative stress biomarkers were evaluated.

Results: The Morris water maze test indicated an enhanced memory in the combination group. Moreover, the combination treatment of liraglutide and memantine resulted in a remarkable reduction in the beta-amyloid protein level in the brain tissue. Neuronal inflammation and oxidative stress biomarkers were significantly reduced, and the levels of antioxidant parameters were enhanced.

Conclusions: The combination of liraglutide and memantine exerts neuroprotective effects and enhances memory and cognitive functions in rats with Alzheimer's disease.

KEYWORDS: Alzheimer's disease; Cognitive function; Glucagon-like peptide-1; Liraglutide; Memantine

1. Introduction

Alzheimer's disease (AD) is one of the most widespread age-related neurodegenerative diseases. AD represents about 60%-70% of the total cases of dementia. Dementia is more accurately described as a syndrome rather than a disease and includes impaired daily activities and language, learning difficulties, and memory loss. The World Health Organization (WHO) estimates that almost 55 million people suffer from dementia, and 10 million cases are diagnosed annually[1].

The pathophysiology of the disease relies on two well-known hypotheses: the amyloid beta plaques cascade hypothesis[2] and

the tau hyperphosphorylation hypothesis, which is correlated with the breakdown of the neuronal cytoskeleton[3]. Neuronal damage occurs due to chronic inflammation in addition to physiological and structural changes in the neurons, which causes biochemical changes in the brain and ultimately results in nerve cell dysfunction and death[4].

Brain tissue and nerve cells are highly dependent on glucose molecules as a source of fuel and energy[5]. Glucose allows brain tissue to function accurately and undergo the necessary structural neuronal changes that permit normal memory functions and provide the required neuronal plasticity[6]. Insulin receptors are found on neurons. Their activation results in dendritic sprouting, the activation of neuronal stem cells, and cell growth, all of which contribute to neuroprotective effects and neuronal repair. In addition, insulin improves brain functions such as attention, cognition, and memory

Significance

Existing research has shown that activation of glucagon-like peptide-1 receptors boosts neuronal repair and enhances learning and memory. This study demonstrates that liraglutide combined with memantine enhances memory and cognitive function in an Alzheimer's rat model by reducing beta-amyloid protein level and oxidative stress as well as enhancing antioxidant activity.

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How to cite this article: Bakhshwin DM, Alhalabi FW, Barasheed MO, Bakhshwin AM, Jamal MH, Bazuhair MA, et al. Neuroprotective effect of liraglutide and memantine in a rat model of Alzheimer's disease. Asian Pac J Trop Biomed 2024; 14(5): 207-214.

Article history: Received 28 December 2023; Revision 8 February 2024; Accepted 8 May 2024; Available online 30 May 2024

formation[7].

On the other hand, neurotransmitter imbalances, such as the imbalance between glutamate excitatory neurotransmitters and acetylcholine (ACh), aid in AD generation. ACh regulates the synthesis and processing of the amyloid precursor protein. The agonist effects of ACh on M1- and M3-subtype receptors reduce the formation of the amyloid precursor protein, which results in behavioral and cognitive dysfunctions[8].

Memantine, an example of an NMDA antagonist, is recommended as the first-line AD monotherapy[9]. Liraglutide is a glucagon-like peptide-1 receptor (GLP-1R) agonist and is known as an “incretin mimetic”. This medication is used mainly as a daily subcutaneous injection in the treatment of type II diabetes mellitus (DM)[10]. It can pass through the blood-brain barrier, where it binds to GLP-1R in the brain tissue, specifically in the neurons, hypothalamus, and hippocampus. GLP-1R activation has the potential to boost neuronal repair and connectivity and enhance amyloid beta clearance, therefore enhancing learning and memory[11]. A multidrug regimen is recommended for the treatment of AD, as it targets different receptors and results in a net of positive outcomes[9]. In the present study, aluminum chloride (AlCl_3) in combination with *D*-galactose (*D*-GAL) was used as the inducing agent of AD[12].

Aluminum accumulates in the hippocampus, where it causes oxidative stress, neurological damage, and neurotoxicity[12]. Additionally, aluminum induces oxidative stress in the brain and impairs the metabolism of the $\text{A}\beta$ protein, resulting in its build-up, which leads to neuronal death and cognitive impairment[12]. In addition, the continuous administration of *D*-GAL induces aging through oxidative stress, which eventually leads to cognitive impairment[13], and the combination of AlCl_3 and *D*-GAL in animals results in the development of AD-like symptoms[13]. The present study was conducted to assess the neuroprotective effects of memantine combined with liraglutide on an AlCl_3 - and *D*-GAL-induced AD rat model.

2. Materials and methods

2.1 Chemicals and reagents

AlCl_3 and *D*-GAL were purchased from Shanghai Huirui Chemical Technology (Hong Kong, China) and dissolved in normal saline to final concentration of 25 mg/0.5 mL, and 15 mg/0.2 mL, respectively. Liraglutide and memantine were purchased from local pharmacy and dissolved in normal saline to final concentration of 0.075 mg/1.5 mL, and 5 mg/0.5 mL, respectively. Amyloid beta protein, rat glucose ELISA kit and oxidative stress and antioxidant biomarkers kits including malondialdehyde (MDA), catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) were obtained from, Inc., (CA, USA). All other chemicals and solvents were of highest grade commercially available.

2.2. Experimental animals and design

Adult male Wistar rats ($n=25$), weighing (200 ± 20) g, were obtained from Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. The animals had a 12:12-hour day and night

cycle and free access to food and distilled water. The animals were separated into five groups with five rats in each group. The selection of five animals per group was determined by careful consideration of the ‘3Rs’ principle (Replacement, Reduction, Refinement) of ethical guidelines. Additionally, previous research indicates that a group size of five can provide adequate statistical power to detect significant differences.

Rats in each group were treated as follows: Group 1 (negative control): rats were treated with 1 mL/kg of normal saline intraperitoneally (*i.p.*) once daily for 10 weeks; Group 2 (positive control group): rats received 100 mg/kg of AlCl_3 orally once daily[14] and 60 mg/kg of *D*-GAL intraperitoneally once daily[13] as inducing agents; Group 3-5 (liraglutide, memantine, liraglutide + memantine): in addition to receiving 100 mg/kg of AlCl_3 and 60 mg/kg of *D*-GAL, rats were treated with liraglutide (300 $\mu\text{g/kg}$ /twice daily by subcutaneous injection)[15], memantine once daily (20 mg/kg in a 2 mL/kg intraperitoneal injection)[16], and memantine (20 mg/kg in a 2 mL/kg intraperitoneal injection once daily) combined with liraglutide (300 $\mu\text{g/kg}$ /twice daily by subcutaneous injection), respectively. Normal saline was used to dissolve the drugs.

2.3. Assessment of spatial learning and memory using the Morris water maze test

The Morris water maze is a behavioral evaluation that is conducted to measure the spatial learning and memory capabilities of rats. A standard Morris water maze procedure was used with minor modifications[17]. For evaluation, a circular pool of water with a diameter of 150 cm and a height of 45 cm was utilized. The tank was filled with water at a temperature of (25 ± 2) °C. Additionally, the tank was demarcated into four equally sized quadrants, which remained consistent throughout the study. Within one of these quadrants, a circular platform with dimensions of 10 cm $\times$ 10 cm was placed.

Briefly, the rats ($n = 5/\text{group}$) received the allocated treatments for 14 d and were trained to find a hidden platform in the water maze for five consecutive days, with four trials/day in 30-minute intervals. The rats were allowed to search for the platform for up to 60 s, after which they were guided to the platform and allowed to stay on it for 20 s. In each trial, the swimming pathway and latency time to locate the platform were recorded using a video-tracking system. In the probe trials measured on day 7, the rats were allowed to swim for 60 s after the hidden platform was removed. The number of times the platform was crossed, the time to first reach the former location of the platform (latency), and the swimming path were recorded.

In theory, animals have the potential to locate a platform by swimming in any direction inside the pool. However, following the initial phase of learning, typical animals demonstrate a rapid acquisition of the ability to swim directly towards the platform. The rapid movement towards the platform serves as an indication of the presence of spatial learning and memory.

2.4. Brain tissue sampling and preparation

When the study was completed, the rats were deprived of food and water for an entire night, anesthetized using 5% isoflurane with a 71% CV/min constant filling rate for 5 min, and then euthanized

through decapitation. The skull was opened with caution, and each rat's entire brain was quickly taken out and cut mid-sagittally into two hemispheres. The hippocampal halves from the rats of each group were used as follows:

They were further equally divided into two parts to be processed. One part was cleaned with isotonic saline, desiccated on a filter paper, weighed, and then immediately homogenized in frozen phosphate-buffered saline at a pH of 7.4. The homogenate was centrifuged at 2000–3000 rpm for 20 min at 4 °C and the supernatant was separated and stored at –20 °C until its usage for biochemical estimations. The other hippocampal halves were kept in 10% neutral-buffered formalin to be fixed and then examined histopathologically after staining with hematoxylin and eosin.

2.5. Histopathological examination

The rat brain hippocampus specimens were initially preserved in 10% formalin for 24 h to ensure proper fixation. Following this, they were rinsed with tap water to remove excess formalin. Sequential alcohol dilutions were used to dehydrate the specimens, and they were subsequently cleared in xylene. The specimens were then embedded in paraffin and placed in a hot air oven at 56 °C for 24 h to ensure the proper infiltration of the paraffin. After the paraffin was embedded, tissue blocks were prepared using paraffin beeswax to facilitate sectioning with a microtome (Leica RM 2135) at a 4 µm thickness. The resulting tissue slices were then placed on glass slides, deparaffinized, and stained with hematoxylin and eosin for histopathological examination under a light microscope (Olympus, Japan).

2.6. Biochemical estimations

2.6.1. Assessment of amyloid β peptide 1–42 ($A\beta$ 1–42)

The ELISA kits were utilized [rat amyloid β peptide 1–42 ($A\beta$ 1–42) (#MBS726579, MyBioSource, CA, USA)]. In this case, the analyzed sample was represented by a homogenate brain. A spectrophotometer (Thermo Scientific, USA) with a wavelength of 450 nm was used to interpret the results. The intensity of the color was measured spectrophotometrically at 450 nm and the results were compared to the standard curve.

2.6.2. Assessment of oxidative stress and antioxidant enzymes

The oxidative stress markers and antioxidant enzyme levels in hippocampal tissue lysates were quantified using ELISA kits for MDA (#MBS268427, MyBioSource, CA, USA), CAT (#MBS726781, MyBioSource, CA, USA), GSH (#MBS265966, MyBioSource, CA, USA) and SOD (#MBS036924, MyBioSource, CA, USA). All procedures were carried out in accordance with the manufacturer's instructions.

2.6.3. Assessment of oral glucose tolerance test (OGTT)

The OGTT was performed on rats that had fasted overnight on the last day of the experiment. Blood was drawn from the tail vein at intervals of 0, 15, 30, 60, and 120 minutes following glucose loading [a 40% glucose solution (2 g/kg body weight) by gavage], placed in serum separator tubes, and allowed to clot for 2 h. This was followed

by centrifugation for 15 min at 3000 rpm. The serum was collected and stored in labeled tubes at –20 °C for further analysis. Glucose in the serum was quantified using an ELISA kit (#MBS7233226, MyBioSource, CA, USA) as per the manufacturer's instructions, with a microplate reader (BioTek®, Synergy HT, USA) at 450 nm.

2.7. Statistical analysis

All the results were interpreted as mean $\pm$ SEM. One-way ANOVA with the Bonferroni and Fisher LSD *post hoc* test for multiple comparisons was used to analyze the results. Graphpad Prism was used for the graphics and data analysis. The results were considered to have significant differences when the *P*-value < 0.05.

2.8. Ethical statement

The research protocol was approved by the Research Ethics Committee of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia (reference No. PH-1442-54) dated August 20, 2020. All the research procedures strictly followed internationally accepted ethical standards.

3. Results

3.1. Effect of liraglutide and memantine on $AlCl_3$ and D-GAL-induced memory deficits

The effect of different treatments on cognition was assessed using the Morris water maze test. An example of the swimming path of each group is shown in Supplementary Figure 1. Figure 1 illustrates the latency period of each group. It showed a reduction of 5.40%, 8.10%, and 18.24% following the administration of liraglutide, memantine, liraglutide combined with memantine, respectively. The combination therapy yielded superior outcomes compared to the administration of individual medications.

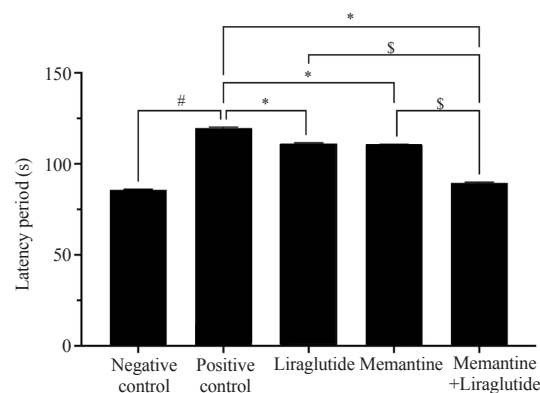


Figure 1. Effect of different treatments on latency period in Morris water maze test. Data are expressed as mean $\pm$ SEM and analyzed by one way ANOVA. **P* < 0.01 compared with the positive control group, #*P* < 0.01 compared with the negative control group, \$*P* < 0.01 compared with the combination group.

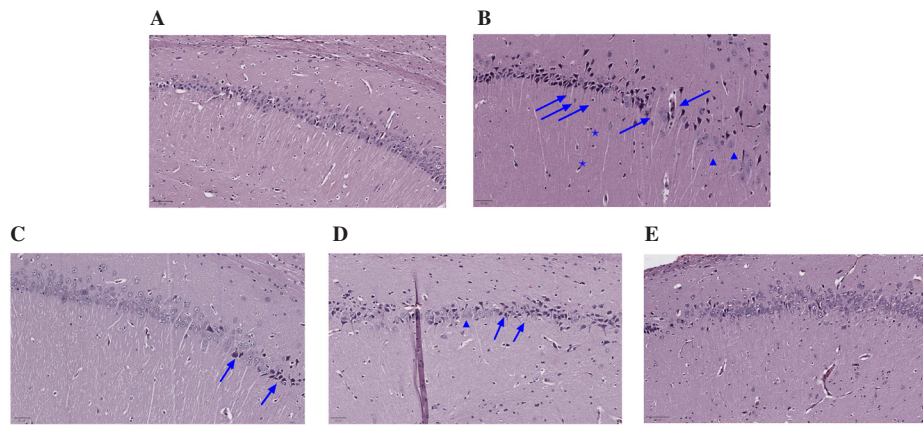


Figure 2. Photomicrographs of the hippocampus: (A) the negative control, (B) the positive control, (C) the liraglutide group, (D) the memantine group, (E) the combination group treated with liraglutide and memantine (H&E, 40×). Arrows: focally deeply stained neurons with pyknotic nuclei; arrowhead: pyramidal neurons; asterisks: reactive neuroglial cells.

3.2. Neuroprotective effect of the combination of liraglutide and memantine on brain histopathology in $AlCl_3$ and D -GAL-induced AD rats

As shown in Figure 2, the negative control sections revealed a typical cornu ammonis of the hippocampus, typified by closely packed pyramidal neurons with large, rounded, vesicular nuclei embedded in a background of unremarkable neuroglial cells and small capillaries. On the other hand, the positive control group displayed an increased number of deeply eosinophilic neurons with pyknotic nuclei along with an apparent decrease in the density of normal pyramidal cells. This was observed on a backdrop of reactive neuroglial cells and congested capillaries. The brain sections obtained from the rats receiving liraglutide and memantine treatments showed deeply stained focal neurons with pyknotic nuclei in a background of normal pyramidal neurons with large, rounded, vesicular nuclei, reactive neuroglial cells, and congested capillaries. Similarly, the combination treatment of both drugs showed mild and rare deeply stained neurons with pyknotic nuclei in a background of normal pyramidal neurons with large, rounded, vesicular nuclei, reactive neurological cells, and congested capillaries. All the examined sections were devoid of abnormal, amyloid-like deposits.

3.3. Effect of the combination of liraglutide and memantine on $A\beta 1-42$ brain level in $AlCl_3$ and D -GAL-induced AD rats

The rats treated with $AlCl_3$ and D -GAL showed a 168.75% elevation in the β -amyloid protein in the brain tissue (Figure 3) as compared to normal rats, which indicates the induction of neurotoxic effects in $AlCl_3$ and D -GAL-treated rats ($P < 0.01$). Treatment with liraglutide, memantine, or their combination reduced the β -amyloid levels of 83.88%, 74.49%, and 62.47%, respectively ($P < 0.01$).

3.4. Effect of the combination of liraglutide and memantine on oxidative stress and antioxidant parameters in the hippocampal tissue of $AlCl_3$ and D -GAL-induced AD rats

A significant decrease in GSH level was observed in the positive control group as compared to the negative control group, while treatment with liraglutide, memantine, or their combination increased $AlCl_3$ and D -GAL-induced reduced GSH level, with the combination therapy showing superior results ($P < 0.01$) (Figure 4A). A similar trend was observed in other antioxidant parameters. The positive control group showed significantly decreased CAT and SOD activities, which were reversed by treatment with both drugs and their combination ($P < 0.01$) (Figure 4C-D). Moreover, MDA level was markedly elevated in the positive control group ($P < 0.01$). All treatments restored MDA to normal level ($P < 0.01$) (Figure 4B).

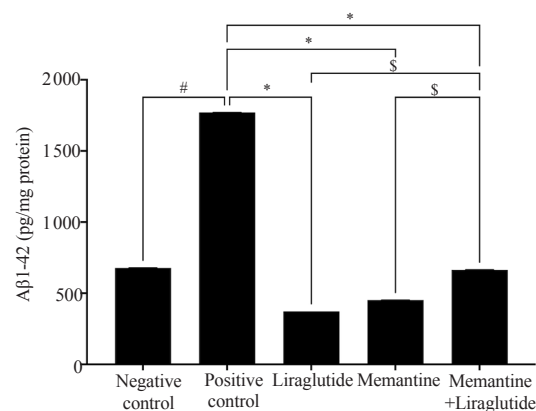


Figure 3. Effect of different treatments on hippocampal $A\beta 1-42$ in rats with $AlCl_3$ and D -galactose-induced Alzheimer's disease. Data are expressed as mean $\pm$ SEM and analyzed by one-way ANOVA with Bonferroni and Fisher LSD *post hoc* test. * $P < 0.01$ compared with the positive control group, # $P < 0.01$ compared with the negative control group, \$ $P < 0.01$ compared with the combination group.

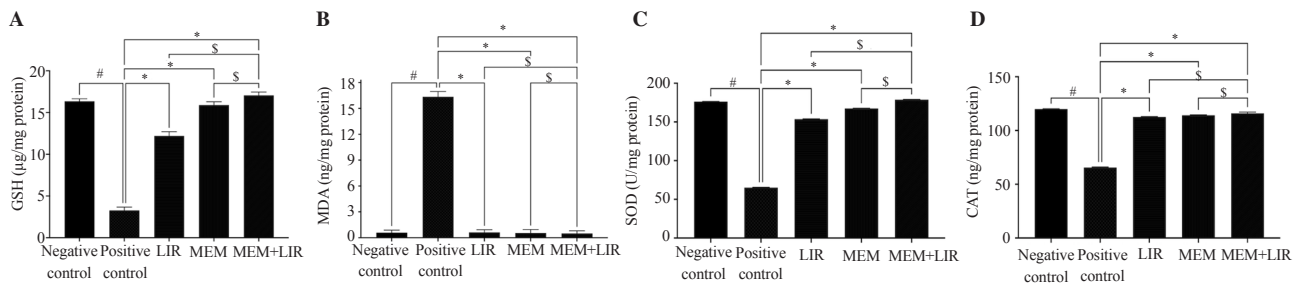


Figure 4. Effect of different treatments on hippocampal (A) GSH, (B) MDA, (C) SOD, and (D) CAT ($n = 5$ per group) in rats with Alzheimer's disease. Data are shown as mean $\pm$ SEM and analyzed by one-way ANOVA with Bonferroni and Fisher LSD *post hoc* test. * $P < 0.01$ compared with the positive control group, # $P < 0.01$ compared with the negative control group, $^{\$}P < 0.01$ compared with the combination group. LIR: liraglutide; MEM: memantine.

3.5. Effect of liraglutide and memantine on OGTT

Figure 5 shows that the fasting plasma glucose levels increased gradually at 15, 30, and 60 min after the glucose gavage in the positive control group, while treatment with liraglutide, memantine, or both drugs attenuated the glucose levels compared with the positive control group.

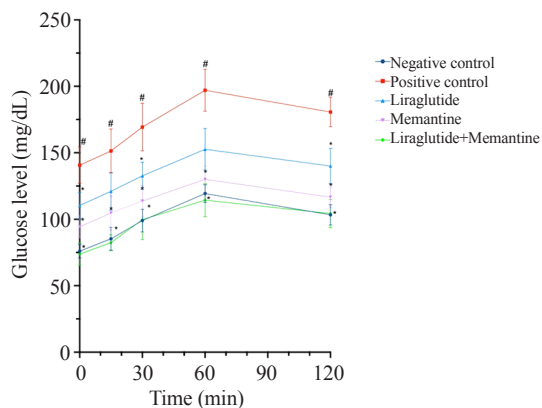


Figure 5. Effect of different treatments on oral glucose tolerance test. Data are expressed as mean $\pm$ SEM ($n = 5$ per group) and analyzed by one-way ANOVA with Bonferroni and Fisher LSD *post hoc* test. # $P < 0.05$ compared with the negative control; * $P < 0.05$ compared with the positive control.

4. Discussion

The existing research indicates that the utilization of a combination of drugs capable of addressing several facets of AD pathology may yield enhanced efficacy in the therapeutic management of AD. According to the existing body of literature, memantine effectively targets abnormalities in glutamatergic transmission[18]. On the other hand, liraglutide has been observed to minimize the impact of neurotoxicity on non-cholinergic mechanisms. It also possesses the ability to manage the maintenance of glucose levels within the body, influence the adaptability of synaptic plasticity, and exhibit actions and impacts that promote the protective effects on neurons[19]. Consequently, a research strategy was designed to investigate the

impact of combining liraglutide and memantine on mitigating the neurotoxic effects caused by AlCl_3 and $D\text{-GAL}$ in rats.

$D\text{-GAL}$ is a pharmacological agent that is recognized for its ability to expedite the aging process[13]. Conversely, aluminum (Al) influences axonal transport and the synaptic structure, resulting in the degeneration of cholinergic neurons. This degeneration manifests as learning difficulties[20]. Previous research has demonstrated that the administration of a combination of AlCl_3 and $D\text{-GAL}$ in rodents can elicit neurodegenerative alterations similar to those observed in AD[21]. These alterations include impairments in the cholinergic system, the upregulation of amyloidogenic proteins, oxidative stress, memory deficits, and cellular depletion in the cerebral cortex and hippocampus. The rats that were subjected to AlCl_3 and $D\text{-GAL}$ in the current study had characteristics that closely mimicked those of normal aging. These traits included memory impairment, hair loss, a humped back, and similarities to AD.

Animal models have demonstrated that metabolic changes can impact and expedite the development of clinical AD characteristics, ultimately leading to cognitive decline[22]. The strong correlation between type 2 diabetes (T2D) and AD provides a basis for investigating the potential of antidiabetic medicines to mitigate or offset AD-related brain pathology and cognitive decline in both animal models and human patients[23]. GLP-1 analogs have been observed to mitigate amyloid and tau diseases, inflammation, and cognitive impairment in many experimental paradigms[11,24,25]. Additionally, initial investigations conducted on human subjects suggest that liraglutide may demonstrate neuroprotective properties in those who are susceptible to AD by enhancing the inherent connection within the brain's default mode network[26].

Currently, memantine is recommended as the first-line AD monotherapy. This drug is a noncompetitive Ca^{2+} channel blocker that binds to NMDA receptors and inhibits Ca^{2+} ion influx into neuronal cells, thereby inhibiting NMDA activation. In addition, it does not interfere with normal physiological activation. In clinical studies, memantine has shown significant outcomes in terms of cognitive function improvement in all stages of AD. In addition to its efficacy, memantine is considered well-tolerated[27].

Takeda and coworkers utilized OGTT to conduct a comparative analysis between individuals diagnosed with AD and those

diagnosed with non-AD dementias. The results of this study revealed a distinct disparity in the plasma levels of A β -40 and A β -42 following glucose administration between AD patients and those with non-AD dementias[28]. Our objective was to investigate the potential of OGTT as a predictive indicator of cognitive impairment[29]. We observed that rats in the positive control group had significantly elevated blood glucose levels as compared to all the treated groups, while the A β 1-42 levels in the brain tissue were also significantly elevated in this group. These changes are in line with earlier reports. In contrast, the combination of liraglutide and memantine significantly restored the OGTT and A β 1-42 levels, thereby demonstrating neuroprotection against AlCl₃ and *D*-GAL-induced neurotoxicity.

Oxidative stress-induced neurotoxicity is a significant clinical characteristic in the underlying neurodegenerative mechanism of AD[30]. Moreover, it is widely acknowledged that oxidative stress is the first occurrence that triggers the development of neurodegenerative disorders and it plays a pivotal role in the cell death triggered by A β in the brain[30]. Dysfunctions related to oxidant levels have been documented in patients with AD, which may be attributable to either the excessive synthesis of oxidants or a deficiency in antioxidants.

GSH, a tripeptide consisting of cysteine, glutamate, and glycine, is a predominant and plentiful endogenous antioxidant within the human body. Research has indicated that the regulation of glutathione homeostasis is disrupted in neurodegenerative disorders[31]. In relation to AD, it has been observed that patients exhibit considerably decreased levels of serum GSH compared to control subjects. Additionally, there is evidence suggesting that the plasma concentration of GSH is associated with cognitive decline and can differentiate between individuals with mild cognitive impairment and healthy volunteers[32]. Elevated concentrations of MDA have been identified as a reliable indicator of oxidative damage in the cerebral tissue of rats. Furthermore, a growing body of evidence derived from both preclinical and clinical investigations indicates a strong association between oxidative stress and the etiopathology of AD[33]. This association encompasses various factors such as mitochondrial malfunction, heightened neurotoxicity mediated by A β , synaptic dysfunction, and neuronal apoptosis[34,35]. The plasma concentrations and enzymatic activity of additional antioxidants, such as extracellular SOD, CAT, and glutathione peroxidase, declined gradually in correlation with the degree of cognitive impairment[36]. Furthermore, a correlation was observed among the levels of lipid peroxides, the existence of senile plaques, antioxidant enzymes, and the growth of neurofibrillary tangles in the brains of individuals with AD[36]. The relationship between oxidative stress and cognitive dysfunction is well established, leading to the belief that pharmacological agents that regulate reactive oxygen species (ROS) play a crucial role in improving cognitive deficiencies[37]. The present investigation observed elevated concentrations of MDA in the cerebral cortex and hippocampus of rats treated with AlCl₃

and *D*-GAL. Nevertheless, all treatments resulted in a decrease in the levels of MDA and an increase in the SOD activity in the brains of rats, indicating the potential antioxidant capabilities of memantine and liraglutide. Moreover, there is evidence suggesting that this combination demonstrates a therapeutic efficacy in mitigating oxidative stress and suppressing A β 1-42 levels, hence ameliorating neuronal cell death. This phenomenon may be attributable to the potential benefits of liraglutide combined with memantine and its concurrent antioxidant properties, which may interact with many signaling pathways involved in the regulation of neuronal survival. Further, to confirm the neuroprotective effect, we performed the Morris water maze test, which involves an animal utilizing distal cues to locate a submerged platform in water. It is often regarded as the most favored behavioral study[17].

The rats in the positive control group exhibited aimless and random movements along the periphery of the pool, in contrast to the rats in the negative control group, which showed a tendency to stay near the platform area and successfully navigate towards it. The findings of this experiment were consistent with those of several earlier investigations, in which animal models treated with AlCl₃ and *D*-GAL exhibited comparable behavior[38,39]. Specifically, these animals tended to stay close to the walls, traveled longer distances, and spent more time before successfully reaching the hidden platform. In other instances, they were unable to locate the platform at all.

These findings suggest the presence of cognitive deficits related to memory and learning. Additional metrics that provided evidence of memory deficits in this study included a notable increase in the escape latency required to reach the platform. Histological alterations indicative of significant neurodegeneration signs were detected in the group treated with AlCl₃ and *D*-GAL. These discovered changes are consistent with previous findings reported in the literature[38,39].

The detected modifications in the liraglutide group align with the findings of Abd El-Rady *et al*[40]. Additionally, the histopathological abnormalities found are consistent with the research conducted by Jadhav and Kulkarni[41]. Further histological findings also demonstrated that the administration of liraglutide in combination with memantine resulted in mild to moderate alterations in the hippocampus tissue. This suggests that the combined use of both medications provides more significant neuroprotection than those observed in monotherapy.

Despite the promising findings of the current study, several limitations must be acknowledged. While the results indicate the potential efficacy of liraglutide as a neuroprotective agent in an AD model, the study lacks a comprehensive evaluation of the side effects and safety profile of liraglutide. Indeed, the 10-week duration may not provide sufficient time to observe the long-term effects or identify possible chronic side effects associated with liraglutide use. Furthermore, the mechanisms underlying the neuroprotective effects of liraglutide remain to be fully elucidated.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgments

The authors acknowledge the Deanship of Scientific Research at King Abdulaziz University, Jeddah, Saudi Arabia for the technical and financial support.

Funding

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant No. G:455-248-1442. The authors, therefore, acknowledge with thanks the DSR for its technical and financial support.

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

Conceptualization, DMB, FWA, ZAD and SMA; Formal analysis, FWA, MOB, AMB, MHJ, MAB and RMM; Investigation, FWA, MOB, AMB, MHJ, MAB, FOK and RMM; Methodology, DMB and FWA; Resources, SMA; Supervision, DMB, ZAD and SMA; Writing – original draft, DMB and FWA; Writing – review & editing, MOB, AMB, MHJ, MAB, FOK, RMM, ZAD and SMA.

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Edited by Liang Q, Tan BJ