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Beneficial Therapeutic Impact of Berberine and Donepezil on Neurotoxicity in Rats

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KEY WORDS

Alzheimer's disease; Berberine chloride; noradrenaline; Donepezil; oxidative stress.

ABSTRACT

Alzheimer's disease (AD) is a neurological ailment is marked by cognitive impairment, oxidative stress, and damage to neurons. Berberine chloride (BBR) is an antioxidant supplement that can help with oxidative stress that comes from brain injury. This study sought to investigate the therapeutic potential of BBR. It also aimed to identify any synergistic effects when paired with donepezil treatment on cerebral injury in rats. We set up seven experimental groups: control group that got saline (GI), control group that got berberine (GII), control group that got donepezil (GIII), $AlCl_3$ /D-galactose-induced AD group (GIV), AD group that got berberine (GV), AD group that got donepezil (GVI), and AD group that got both berberine and donepezil (GVII). We looked at biochemical markers such catalase activity, levels of noradrenalin, liver and kidney function, lipid profile, and calcium levels. A histopathological study was conducted to assess neuronal integrity. The AD model group showed significant decrease in catalase, noradrenalin, albumin and HDL as well as significant increase in liver enzymes, urea, creatinine and calcium concentration with serious damage to neurons in the hippocampus. Berberine or donepezil alone partially corrected these changes, making the antioxidant defense, neurotransmitter balance, and tissue morphology better. The combination therapy notably yielded the most significant reinstating biochemical and morphological change in hippocampus beyond the effects of either treatment alone. In conclusion, BBR exhibited significant neuroprotective and its conjunction with donepezil had synergistic effects against Alzheimer's disease-like pathology. These findings indicate that berberine presenting a viable approach for the management of AD.

Introduction

Significant neuro inflammation, synapse disruption, and notable effects on nervous system functions are characteristics of pathological disorders referred to as neurodegenerative illnesses (**Elariny et al., 2024**). For cells and neurons to function properly, the metal ion homeostasis in the brain is essential, and when it is disturbed, neurodegeneration progresses. According to **Mohapatra et al. (2022)**, a variety of metals like copper, mercury, zinc, aluminum, lithium, lead, silica, and iron, can be neurotoxic. Aluminum and other common metals can cause neurodegenerative diseases and cognitive problems by increasing oxidative stress, lowering cholinergic neurotransmission, and inflaming neurons. Aluminum mostly passes the blood-brain barrier and builds up in the cortex, hippocampus, and cerebellum of the brain, causing a sickness that is similar to AD **(Alqhtani, 2025).** D-galactose (D-gal), a reducing sugar, interacts with the amino groups in proteins to make advanced glycation end products (AGEs), which hasten the aging process of cells. Chronic low-dose D-gal administration results in aging-like symptoms in animals, such as oxidative stress, immune system weakening, altered gene expression, and cognitive impairment. These characteristics make D-gal a popular experimental model for drug screening and AD research. According to **Chiroma et al. (2018)**, it has been shown to significantly raise the levels of acetyl cholinesterase (AChE) in mice's brains, a sign of cholinergic dysfunction in AD. D-galactose induces several clinical manifestations, including oxidative stress, inflammation, compromise of the intestinal barrier, and diminished cognitive and motor functions **(Zhou et al., 2025).**

Mitochondrial dysfunction is a significant contributor to oxidative stress in the brain. Neural cells are particularly susceptible to oxidative stress due to their elevated metabolic activity and dependence on aerobic respiration, which generates reactive oxygen species (ROS) as byproducts. **Trofin et al. (2025)** contend that heightened generation of ROS can result in lipid peroxidation, protein oxidation, and DNA damage, all of which compromise neuronal integrity and survival.

AD characterized by a continuous deterioration in synaptic and neuronal activity, impedes memory and cognitive processes. The accumulation of amyloid beta peptides (Aβ) and intracellular neurofibrillary tangles in neuronal cells is considered the primary histological hallmark of AD **(Oyagbemi et al., 2025).** The breakdown of acetylcholine impacts the awareness of AD patients, whereas acetyl cholinesterase treatments enhance memory function. The neurotransmitter scales at the synapse are getting bigger **(Walczak-Nowicka & Herbet, 2021).** The FDA has authorized three cholinesterase inhibitors: donepezil for all stages of AD, and galantamine and rivastigmine for mild to moderate AD **(Haake et al., 2020).**

Berberine chloride (BBR) is an alkaline isoquinoline often located in the Berberidaceae family's genus Berberis **(Hashmi et al., 2024).** Berberine is usually sold in tablet or capsule form as salts like berberine sulfate and berberine chloride since it doesn't dissolve in water. Numerous therapeutic effects, including

anti-inflammatory, anti-oxidative stress, and neuroprotective advantages, have been shown **(Kim et al., 2022).** Berberine had a neuroprotective effect in chronic cerebral hypoperfusion, indicating its potential therapeutic use for conditions such as vascular dementia **(Begh et al., 2025).**

Numerous studies have shown that berberine can reduce the evolution of AD in numerous ways, such as by preventing the creation of Aβ and the excessive phosphorylation of Tau protein. Berberine inhibits acetyl cholinesterase and other significant enzymes implicated in the pathogenesis of AD **(Yuan et al., 2019).**

Aim of study

The objective of this study was to evaluate the neuroprotective and therapeutic effects of donepezil and berberine chloride (BBR) on brain injury in rats induced by D-galactose and $AlCl_3$. It also examined any potential synergistic effects of the two medications. The study aimed to comprehensively evaluate the efficacy of berberine both independently and in conjunction with donepezil by examining oxidative stress markers, neurotransmitter levels, hepatic and renal function, lipid profiles, calcium concentrations, and hippocampal structure.

Materials and Methods

Chemicals and drugs

$AlCl_3$ and D-Galactose (D-gal) white crystals were obtained from Sigma-Aldrich, USA. Berberine chloride powder was purchased Thermo Scientific Alfa Aesar L03807.14 and donepezil tablet was obtained from Delta pharma (DP) Co., (Egypt).

Animals

Seventy white male albino rats, 6-7 weeks old and weighting 170-180 g was used in the experimental investigation of this study. This experiment was carried out and accepted by the Animal Ethics Committee (3-12-22) in compliance with Egyptian ethical codes for research on laboratory animals, Damanhour University. The rats became used to living in wire mesh cages for two weeks. They were given regular store-bought meals and free access to water. The rats were kept in a room that was around 25 ±2°C and had a 12:12-hour light-dark cycle.

Design of the Experiment

The rats were divided to seven groups, ten rats in each group: GI (Normal control): Normal healthy rats take only saline. GII (Berberine chloride control): Normal healthy rats were administrated orally by Berberine chloride at a dose of 50 mg/kg daily for 14 days **(Zhu & Qian, 2006)**. GIII (donepezil control): Normal rats were administrated donepezil orally with a dose of 10 mg/kg daily for 14 days **(Meier-Davis et al., 2012)**. GIV (AD model): rats were intraperitoneally injected with D-galactose at a dose of 120 mg/kg daily for 30 days and daily oral administration of $AlCl_3$ at a dose 50 mg/kg to 4 weeks **(Talib et al., 2025)**. GV (AD treated with Berberine Chloride): AD model given berberine chloride by mouth at a dosage of 50 mg/kg per day for 14 days. GVI (AD treated with donepezil): An AD model was given donepezil at a dosage of 10 mg/kg every day for 14 days. GVII (AD treated with both donepezil and berberine chloride): The AD model was treated with donepezil at a dose of 10 mg/kg and berberine chloride at a dose of 50 mg/kg daily for

14 days. At the conclusion of the experiment, the rats were euthanized, and blood samples were obtained in plain tubes. The hippocampus was subsequently cut into two sections: one was preserved at -80°C for biochemical analysis, while the other was fixed in 4% paraformaldehyde for histological inspection. Table (1) below shows a summary of the experimental plan.

Table (1): Experimental design and drug doses in different groups of rats

Groups	Names of groups	Compound and drugs administered
GI	Normal control	Normal saline.
GII	Berberine chloride control	Berberine chloride (50mg/kg daily for 14 days).
GIII	Donepezil control	Donepezil (10 mg/kg daily for 14 days).
GIV	AD model	D-galactose (120 mg/kg daily) + $AlCl_3$ (50 mg/kg) to 4 weeks.
GV	AD treated with Berberine Chloride	D-galactose (120 mg/kg daily) + $AlCl_3$ (50 mg/kg) to 4 weeks then berberine chloride (50 mg/kg daily for 14 days).
GVI	AD treated with donepezil	D-galactose (120 mg/kg daily) + $AlCl_3$ (50 mg/kg) to 4 weeks then donepezil (10 mg/kg daily for 14 days).
GVII	AD treated with both donepezil and berberine chloride	D-galactose (120 mg/kg daily) +$AlCl_3$ (50 mg/kg) to 4 weeks then berberine chloride nanoparticles (50 mg/kg) + donepezil (10 mg/kg) with each other for 14 days.

Preparation of hippocampus homogenate

Before homogenizing the tissues in nine volumes of ice-cold 0.05 mM potassium phosphate buffer (PH 7.4) with a glass homogenizer to make 10% homogenates, they were cut, weighed, and chopped into small pieces. **Raina et al. (2025)** say that the homogenates were spun for 15 minutes at 6000 r.p.m. at 4°C.

Determination of Catalase and Noradrenaline

Hippocampus noradrenaline was estimated via ELISA-kit (Eagle Biosciences, Cat. No: NOU39-K01). Hippocampus CAT activity (EC .1.11.1.6) was measured by the colorimetric technique using available commercial kit (Bio diagnostics Cat. No CA 25 17) accordance with the method of (**Aebi, 1984**).

Determination of blood glucose and Calcium level

Fasting glucose was measured by colorimetric assay using available commercial kit (Vitro scient- Diagnostics, Egypt, Cat. No. GL 118 08) described by (**Trinder, 1969**). Serum Calcium level was detected as claimed by colorimetric method using commercial kit (Bio Diagnostics, Egypt, Cat. No CA 12 10) represented by (**Gindler and King, 1972**).

Determination of liver and kidney function

Serum alanine aminotransferase (EC 2.6.1.2) activity and Serum aspartate aminotransferase) (EC 2.6.1.1) activity was evaluated by the kinetic method using available commercial kit (Vitro scient- Diagnostics, Egypt, Cat. No. AL 10 31) and (Vitro scient- Diagnostics, Egypt, Cat. No. AS 10 61) respectively. These assay system was critically estimated and adjusted by (**Schumann et al., 2002)**

Serum Albumin was detected by the Colorimetric endpoint assay by modified bromcresol green binding assay (BCG) **(Rodkey, 1964).** The test was done by

using (Vitro scient- Diagnostics, Egypt, Cat. No. AB 10 10)

Serum urea concentration was assessed by using the commercial kit (Vitro scient- Diagnostics, Egypt, Cat. No. UR 21 10) according to (**Malhotra, 2003**). As well as serum creatinine concentration was evaluated by utilizing the commercial kit (Vitro scient- Diagnostics, Egypt, Cat. No. CR 12 50) described by (**Chary and Sharma, 2004).**

Determination of lipid profile

Serum triglycerides were detected according colorimetrical assay by commercial kit (Vitro scient- Diagnostics, Egypt, Cat. No. TR 20 30) reported by **(Fossati & Prencipe, 1982).** Serum total cholesterol concentration was estimated using the commercial kit (Vitro scient- Diagnostics, Egypt, Cat. No. CH 12 20) according to **(Allain et al., 1974).** As well as serum HDL-C concentration was determined by addition of phosphor tungstic acid and magnesium chloride is utilized to sediment chylomicrons, VLDL (very low density lipoproteins) and LDL (low density lipoproteins). After centrifugation the supernatant consist the HDL. The test was done by using commercial kit (Vitro scient- Diagnostics, Egypt, Cat. No. CH 12 30) (**Gordon et al., 1977)**. Serum LDL was detected by the following equation:

LDL-cholesterol = TC – TG/5 – HDL-cholesterol **(Falholt et al., 1973).**

Histopathological investigation (HE staining)

The hippocampal tissue was removed and kept in 4% paraformaldehyde in PBS (0.1 M, pH 7.4 PBS) for 24 hours at 4°C. The fixed hippocampal tissue was dried using a succession of ethanol solutions of different strengths and then put into paraffin according to normal methods. We utilized glass slides covered in gelatin chromalum to retain paraffin slices that were 5 μm thick so we could stain them with hematoxylin-eosin **(Beltagy et al., 2024).**

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS software, version 16) was used to look at the data statistically. It showed the mean and standard deviation (SD) of the data. They performed the one-way ANOVA (Tukey) test to identify big differences between the means. If the P value was lower than 0.05, it was statistically significant **(Izzularab et al., 2022).**

Results

Findings of oxidative stress marker and neurotransmitter

The hippocampal homogenates of AD rats (GIV) showed a significantly significant decrease in catalase activity (CAT) as compared to normal control (GI). In addition, all treatment groups (GV, GVI, and GVII) exhibit a considerable rise in CAT as compared to GIV. Catalase activity's precise numerical values are listed in Table 2.

Following treatment with $AlCl_3$ and D-gal (GIV) compared to GI, the hippocampal noradrenalin level significantly decreased, according to the study's data, which are shown in Table 2. However, compared to rats treated with D-gal and $AlCl_3$ (GIV), treated animals (GV, GVI, and GVII) showed a substantial increase in hippocampal noradrenalin levels.

Fasting Glucose and Serum calcium ion measurement

When compared to normal control (GI), the serum of AD groups (GIV) showed a marked rise in both blood glucose and Ca^{+2} levels. When compared to GIV, the

results indicated a significant regression in both blood glucose and Ca^{+2} concentrations in all treated groups (GV, GVI, and GVII). The studies' specific numerical values are present in Table (3).

Liver and kidney function

The study demonstrated that GIV had markedly elevated levels of urea and creatinine, along with considerably increased ALT and AST activity compared to GI. But GIV has a lot less albumin than GI. After therapy, both GV and GVII had substantially lower levels of ALT and AST activity, as well as much lower levels of urea and creatinine than GIV. Also, the concentration of albumin is significantly higher than it was in GIV. Both GIII and GVI, which were treated with donepezil medication, show higher levels of ALT and AST activity and higher levels of urea and creatinine compared to GI and GIV, respectively. The results indicate a considerable drop in albumin concentration in GIII compared to GI, and a large rise in albumin concentration in GVI compared to GIV.

Lipid profile

Rats who got daily shots of (D-gal) and ($AlCl_3$) for four weeks had much lower HDL levels in GIV than in GI, but much higher CHO, TG, and LDL levels in GIV than in GI. Furthermore, in comparison to GIV, GV and GVII exhibited much lower levels of CHO, TG, and LDL. Table (5) shows that GV and GVII also had considerably greater HDL than GIV. GVI that was treated with donepezil still shows bad effects.

Table (2): Antioxidant enzyme (CAT) and neurotransmitter (Noradrenaline) in different groups.

Groups	CAT (U/g.tissue)	Noradrenaline (ng/g.tissue)
GI	256.4±4.8	13.4±0.3
GII	254.1±3.4	13.2±0.2
GIII	255.6±5.6	12.8±0.1
GIV	156.8±4.1[a]	7.1±0.3[a]
GV	266.0±2.4[a,b]	8.6±0.2[a,b]
GVI	209.1±4.7[a,b]	12.1±0.08[a,b]
GVII	241.4±7.1[a,b]	12.9±0.05[a,b]

Data are presented as mean ± S.D, n=10 per group. P value (P≤ 0.05) is significant; a: Significant VS. Control group (GI), b: Significant VS. AD model (GIV).

Table (3): glucose level and Calcium ion detection in different groups.

Groups	Glucose (mg /dl)	Calcium (Ca^{+2}) (mg /dl)
GI	111.1± 4.2	8.0 ± 0.9
GII	110.5±1.6	8.4 ± 0.7
GIII	111.0 ± 1.3	8.9 ± 0.9
GIV	222.4 ± 4.3[a]	20.8 ± 2.0[a]
GV	182.6 ± 3.9[a,b]	12.4 ± 1.1[a,b]
GVI	198.7 ±3.6[a,b]	16.1 ± 1.3[a,b]
GVII	126.4 ± 1.7[a,b]	13.2 ± 1.0[a,b]

Data are presented as mean ± S.D, n=10 per group. P value (P≤ 0.05) is significant; a: Significant VS. Control group (GI), b: Significant VS. AD model (GIV).

Table (4): Serum analyses in different groups include (ALT, AST, albumin, urea and Creatinine levels) in different groups.

Groups	ALT(U/L)	AST(U/L)	Albumin(g/dl)	UREA (mg/dl)	Creat (mg/dl)
GI	19.7±1.1	25.0±0.7	3.3±0.04	17.1±0.7	0.73±0.04
GII	18.9±0.7	24.8±0.7	3.3±0.03	17.0±0.3	0.68± 0.08
GIII	21.1±0.5 [a,b]	26.1±0.6[a,b]	3.0±0.05[a,b]	19.4±0.3[a,b]	0.91±0.05[a,b]
GIV	50.4±1.2[a]	51±0.7 [a]	2.5±0.03[a]	45.8±1.5[a]	2.2±0.06[a]
GV	41.0±0.7 [a,b]	42.6±0.6 [a,b]	3.1±0.04 [a,b]	31.6± 0.4 [a,b]	1.6± 0.03 [a,b]
GVI	55.0±0.7 [a,b]	57.0±0.7 [a,b]	2.6±0.04 [a,b]	56.4± 1.7 [a,b]	2.5± 0.04 [a,b]
GVII	29.8±1.9 [a,b]	33.3±2.9 [a,b]	3.0±0.04 [a,b]	34.2± 1.1 [a,b]	1.4± 0.03 [a,b]

Data are presented as mean ± S.D, n=10 per group. P value (P≤ 0.05) is significant; a: Significant VS. Control group (GI), b: Significant VS. AD model (GIV).

Table (5): Serum lipid profiles include (HDL, LDL, CHO, and TG) levels in different groups.

Groups	CHO(mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dl)
GI	147±2.2	45.1±2.4	58.7±1.9	79.2±2.0
GII	144±2.2	43±1.7	60.1±2.4	75.3±2.2
GIII	153±1.5 [a,b]	48.2±0.4[a,b]	52.4±1.4[a,b]	91±1.3[a,b]
GIV	289.5±3.4[a]	88.5±4.0 [a]	30.6±2.0[a]	241.2±2.9[a]
GV	249±3.2 [a,b]	77±6.5 [a,b]	44.4±2.1 [a,b]	189.2±2.6[a,b]
GVI	295.3±4.6 [a,b]	94±4.7 [a,b]	26.4±1.2 [a,b]	250.1±2.9 [a,b]
GVII	259.5±7.1 [a,b]	79.3±4.9 [a,b]	40±2.9 [a,b]	203.6±5.9 [a,b]

Data are presented as mean ± S.D, n=10 per group. P value (P≤ 0.05) is significant; a: Significant VS. Control group (GI), b: Significant VS. AD model (GIV).

Histopathological findings of hippocampus tissue

Histological research illustrated coronal slices of the hippocampal microstructure across several study groups, stained with hematoxylin and eosin. The three hippocampus areas in the control groups showed no alterations in histology (Fig. 1A–C). However, slices of the hippocampus from rats administered D-galactose and $AlCl_3$ (GIV) exhibited a significant number of injured neurons characterized by apoptosis, vacuolated astrocytes, and pervasive vacuolar degeneration, with a reduction and deformation of the pyramidal cells Fig. (1 D & E). Nonetheless, a significant enhancement was evident in the treated cohorts Fig. (1 F&G), particularly in GV and GVI), wherein hippocampal sections administered donepezil and berberine chloride for AD exhibited mild atrophy, mild diffuse vacuolar degeneration, and a rise in pyramidal cells (Fig. 1 H) in GVII.

Discussion

Klyucherev et al. (2022) assert that Neurodegeneration, characterized by neuronal atrophy and/or loss is linked to toxic amyloid-beta oligomers and protein aggregates, intra-neuronal neurofibrillary tangles formed by hyperphosphorylated microtubule-associated protein Tau, regionally specific reductions in cerebral glucose metabolism, synaptic impairment, and cardiac dysfunction. Aluminum (Al), a neurotoxic substance in the brain, is responsible for the formation of amyloid aggregates and neurofibrillary tangles **(Dey and Singh, 2022).** Aluminum has been shown to compromise neurological function by causing Aβ degradation in brain tissue, cellular apoptosis, and manifestations akin to AD **(Tzioras et al., 2023).** The diagnosis and treatment

of AD thus depend significantly on memory and learning capabilities. **Asghar et al. (2015)** assert that exposure to $AlCl_3$ generates perilous intermediates, including hydrogen peroxide and hydroxyl radicals, which may mitigate the toxicity of aluminum. Prolonged administration of low levels of D-gal has been associated with alterations in gene transcription, oxidative stress, cognitive deterioration, and compromised immunological function. These are all indicators of typical aging in animals. Prolonged systemic administration of D-gal in rats often leads to neurotoxicity, providing a model for examining the processes behind AD **(Haider et al., 2020).**

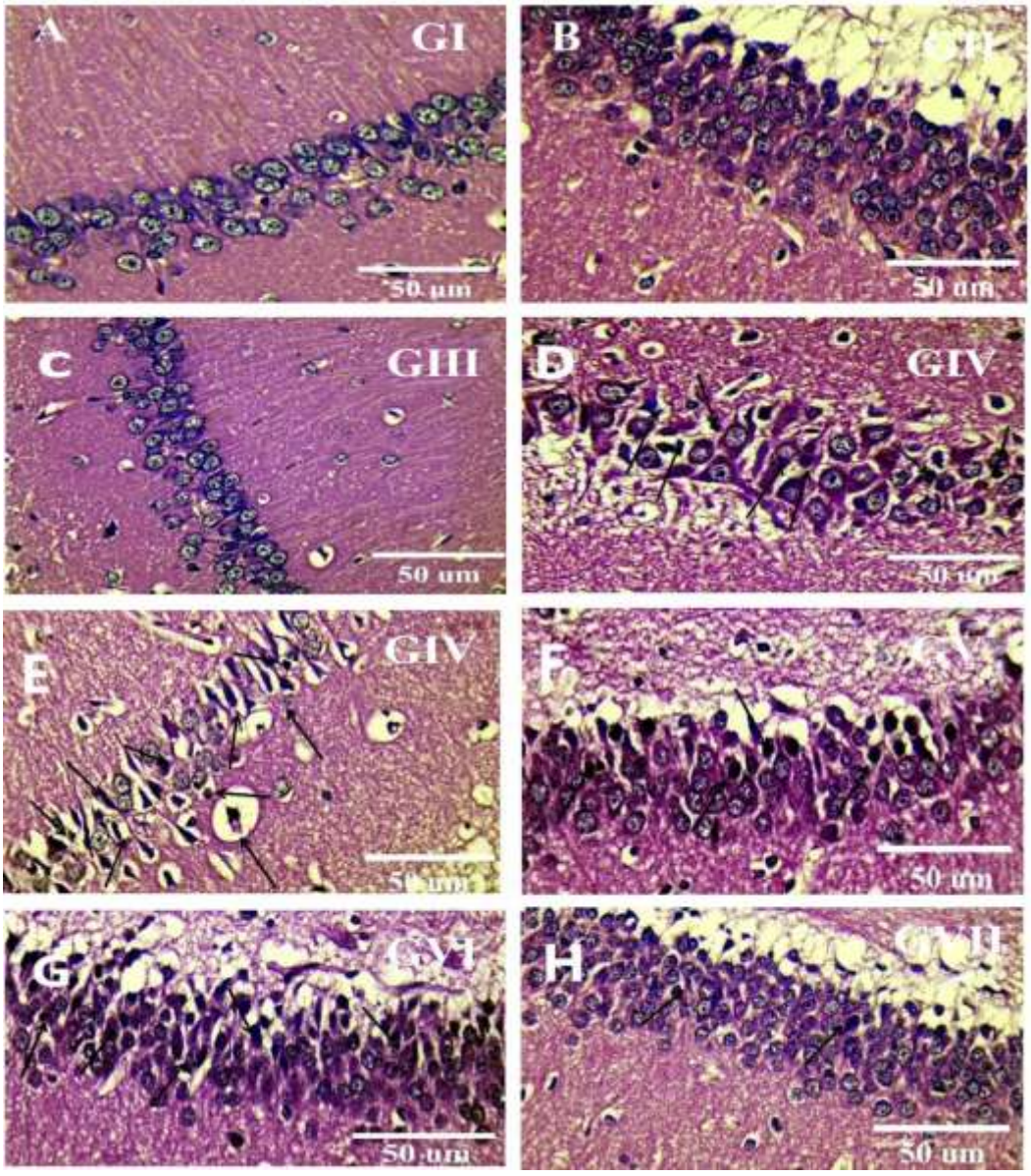

Fig. (1): Photomicrograph of the rat stained with Haematoxylin and Eosin. A&B&C: GI, GII, and GIII showed that the pyramidal cells, astrocytes, and fibers were all in their usual shape and structure. D&E: Sections of the hippocampus in GIV showed a lot of damaged neurons, pyramidal cells that had died off and reduced in number (arrows), and astrocytes that had vacuoles. F&G: Hippocampus slices from GV and GVI revealed a little rise in the number of pyramidal cells and a small improvement in the health of damaged neurons. H: Parts of the GVII hippocampus tissue revealed some extensive vacuolar degeneration, some atrophy, and more pyramidal cells.

Previous studies have demonstrated that rats subjected to chronic administration of D-gal or $AlCl_3$ exhibited changes resembling typical aging processes, such as oxidative stress, cognitive deterioration, and cholinergic system dysfunction **(Kumar et al., 2011). Chiroma et al. (2018)** report that rats concurrently treated $AlCl_3$ and D-gal exhibited signs of cognitive deterioration and degeneration of pyramidal cells in the hippocampus. This study demonstrated that rats treated $AlCl_3$ with D-gal had significant learning difficulties. Oxidative stress-induced neurotoxicity is a major pathogenic factor in the neurodegenerative pathways of AD. Oxidative stress is crucial for Aβ-induced neuronal cell death and is frequently considered a precursor to neurodegenerative diseases **(Buccellato et al., 2021).** The initial discovery of berberine (BBR) originated from the bark plant Xanthoxylon clava **(Verma et al., 2023).** Plants belonging to the families Papaveraceae, Loganiaceae, Rutaceae, Menispermaceae, Rhamnaceae, and Berberidaceae have previously demonstrated the presence of BBR. Individuals may currently synthesize BBR in a laboratory setting **(Zhang et al., 2025).** Numerous investigations on the pharmacological effects and associated mechanisms of BBR have been undertaken in various countries in recent years. These investigations demonstrate that BBR has unique effects and activities on the cardiovascular, endocrine, and neurological systems. These activities can lower blood lipids and safeguard the cardiovascular system by altering neurotransmitters in the brain. They can also improve insulin sensitivity in individuals with diabetes and alleviate anxiety. The potential applications of BBR in the prevention and treatment of neurological, cardiovascular, and cerebrovascular illnesses are difficult to overlook **(Sunhe et al., 2024).** Our findings demonstrated a 2.59-fold increase in calcium levels in the AD model compared to the normal control group **(Nawar et al., 2025)**. The minerals are essential for the proper functioning of the central nervous system. An elevated risk of neurological illnesses may be linked to aberrant increases or decreases in brain concentrations **(Dibacto et al., 2022).** Progressive neuronal degeneration, calcium ion dysregulation, and metabolic irregularities, particularly mitochondrial dysfunction, are pathogenic characteristics of AD. Mitochondria may lose their capacity to regulate calcium efficiently. **Ashleigh et al. (2023)** assert that Aβ creates pores in the plasma membrane, facilitating the influx of calcium and resulting in its accumulation in the cytosol. This may result in mitochondria absorbing excessive calcium, which can subsequently induce the formation of free radicals and impair mitochondrial function. Calcium ions (Ca^{2+}) regulate several neuropathological processes via specialized transporters. An imbalance of Ca^{2+} in AD disrupts the control of Aβ, tau, and neural plasticity, specifically regarding Ca^{2+} transporters in cellular, mitochondrial, endoplasmic reticulum (ER), and lysosomal membranes **(Guan et al., 2021).** Excessive calcium accumulation in mitochondria activates neuronal nitric oxide synthase (nNOS), leading to the production of nitric oxide (NO) and the formation of peroxynitrite (ONOO) with O_2, resulting in oxidative stress due to elevated (Ca^{+2}) levels **(Huang et al.,**

2018). Al^{+3} inhibits calcium (Ca^{+2}) metabolism by blocking Ca^{+2} channels and negatively impacting Ca^{+2} signaling pathways. **Breijyeh and Karaman (2020)** assert that it competes with this cation for small ligands such as phosphates. Relative to the AD group (GIV), calcium levels in all treatment groups (GV, GVI, and GVII) increased by roughly 0.59, 0.77, and 0.63 times, respectively. Mitochondrial Ca^{2+} overload is widely recognized as a consequence of excessive Ca^{2+} influx into the mitochondria. The permeability transition pore (PTP) opens due to this overload, resulting in mitochondrial swelling and ultimately cellular demise. Berberine possesses a well-established safety profile corroborated by several clinical and preclinical studies. It significantly influences mitochondrial Ca^{2+} uptake **(Zhao et al., 2025).** The previously published glucose data is clarified by the association between more severe plaques and tangles in Alzheimer's brains, which are connected to increased brain glucose levels and reduced glycolytic rates. The conducted investigation revealed that the blood glucose concentration in the AD model was nearly double that of the normal control group. In the GV, GVI, and GVII groups, blood glucose levels were significantly lower than in the AD group, by about 0.82, 0.89, and 0.56 times, respectively. **Yang et al. (2025)** and **Piotrowska et al. (2025)** contend that BBR alleviates hyperglycemia, substantiating their results. BBR serves as a viable alternative to conventional pharmacological therapies by enhancing insulin efficacy, reducing blood glucose levels, and activating AMP-activated protein kinase (AMPK). It also facilitates enhanced regulation of glucose metabolism **(Jin et al., 2017). Nawar et al. (2025)** contend that these findings correspond with the hypothesis that elevated blood glucose levels, considered normal, may be associated with the etiology of AD. Amyloid beta-derived diffusible ligands that disrupt insulin signaling may induce a kind of diabetes that only impacts the brain, resulting in insulin resistance inside the AD brain **(Kandimalla et al., 2017).** The findings demonstrate that the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the AD model were significantly increased relative to the normal control group **(Nho et al., 2019; Nawar et al., 2024).** The serum ALT and AST levels in the AD model rats were significantly elevated relative to the normal control group, as D-gal overload can augment the generation of ROS, leading to oxidative stress that damages critical cellular components, promotes lipid peroxidation, disrupts the membranes of cells and organelles in the liver, and induces hepatocyte swelling and necrosis **(Estrada et al., 2019).** Increased ALT levels were linked to greater retention of Aβ, suggesting a possible relationship between Aβ pathology and mild liver illness. The liver, a crucial peripheral organ for metabolic detoxification, can modify the dynamic equilibrium between Aβ deposition in senile plaques and soluble Aβ by removing circulating Aβ. The low-density lipoprotein receptor-related protein 1 (LRP-1), highly active in hepatocytes, may facilitate the hepatic uptake of circulating Aβ **(Kim et al., 2025).** The preliminary study's results demonstrated that the AST level in the AD model was around 2.04 times higher than that of the normal control group.

Compared to the AD group, the AST levels in the treated groups (GV, GVI, and GVII) increased by approximately 0.83, 1.11, and 0.65 times, respectively. The preliminary study's results demonstrated that the ALT level in the AD model was significantly increased by around 2.55 in comparison to the normal control group. The ALT levels rose by about 0.81, 1.09, and 0.59 times when comparing the GV, GVI, and GVII groups to the AD group, respectively. **Erbayraktar et al. (2017)** reported that **donepezil** treatment increased both ALT and AST levels. Rats administered (BBR+Don) exhibited the most significant improvement in their ALT and AST levels. According to **Nie et al. (2024),** BBR effectively reduces ALT and AST levels. The concentration of pure Aβ in the circulation may remain constant due to the continual binding and transport of Aβ by albumin. Thus, we hypothesized that a buildup of Aβ in the brain may result from a reduced ability of the brain to discharge Aβ into the circulation, linked to decreasing blood albumin levels **(Yamamoto et al., 2014)**. The blood albumin levels in the AD model group were 0.73 times lower than those in the normal control group. The albumin levels in the GV, GVI, and GVII groups exceeded those in the AD group by approximately 1.24, 1.06, and 1.22 times, respectively. The findings of the current study indicate that blood urea and creatinine levels in the AD model were significantly elevated compared to the normal control group, by about 2.67 and 3.02 times, respectively. These findings correspond with those of **Nawar et al. (2024),** who documented markedly increased serum creatinine levels among several age cohorts of AD patients. Mice were given D-gal at a dosage of 120 mg/kg body weight for four weeks, leading to a notable rise in creatinine and urea levels, as well as heightened oxidative stress and nephropathy, following a daily oral administration of 50 mg/kg body weight $AlCl_3$ **(Feng et al., 2016).** This investigation indicated that the administration of donepezil exacerbated the stress on renal and hepatic functions. This finding may arise from the drug's hepatic glucuronidation and renal excretion metabolism. **Erbayraktar et al. (2017)** contend that donepezil may exacerbate impaired liver and renal function, may be due to amyloid buildup in these organs. Upon comparison of the GV, GVI, and GVII groups with the AD group, the levels of urea and creatinine exhibited improvements of around 0.68, 1.23, 0.74 and 0.70, 1.11, and 0.63 times, respectively. Our findings validated previous research **(Kuo et al., 1998; Bonarek et al., 2000; Lesser et al., 2011)**, indicating a substantial increase in serum CHO, LDL, and TG levels, coupled with a decrease in HDL levels in the AD group relative to the Control group. The research findings indicated that the CHO level in the AD model was significantly raised, about 1.96 times higher than that of the normal control group. The CHO level rose by approximately 0.86, 1.02, and 0.89 times in the GV, GVI, and GVII groups, respectively, in comparison to the AD group. Our investigation revealed that the TG and LDL levels in the AD model were significantly elevated compared to the normal control group, by approximately 1.96 and 3.04 times, respectively. Relative to the AD group, the TG and LDL levels in the GV, GVI, and GVII groups improved by approximately 0.87, 1.06, 0.89, and 0.78,

1.04, 0.84 times, respectively. Our study demonstrated a considerable reduction in HDL levels in the AD model; about 0.52 times lower than the normal control group. In comparison to the AD group, HDL levels in the GV, GVI, and GVII groups increased by roughly 1.45, 0.86, and 1.31 times, respectively. BBR markedly reduced lipid production. Fatty acid absorption was also limited by reduced triglyceride synthesis and fatty acid oxidation **(Yu et al., 2021).** According to **Nie et al. (2024)**, BBR significantly enhances lipid profile assessments. Coronal slices of hippocampal microstructure stained with hematoxylin and eosin from several research groups. The hippocampus, a principal area of rat brains situated underneath the cerebral cortex, is characterized by a slender layer of densely packed pyramidal cells, encircled by an inner polymorph layer and an outer plexiform layer. The hippocampus comprises three principal subdivisions: CA1, CA2, and CA3, in that sequence. Due to its layer of well-aligned pyramidal cells, CA1 is readily distinguishable in H&E-stained slices. The CA2 and CA3 regions of Ammon's horn are characterized by large pyramidal cells that form many tightly arranged layers superimposed over one another. No histological changes were seen in the CA1, CA2, and CA3 hippocampal slices of the control group, and these changes diminished after treatment. The cohort that received both berberine chloride and donepezil exhibited the most significant enhancement. Rats administered D-galactose and $AlCl_3$ in the AD model exhibited multiple damaged neurons, characterized by diminished and deformed pyramidal cells, vacuolated astrocytes, apoptosis, and evident widespread vacuolar degeneration. Comparable results indicated that D-galactose and $AlCl_3$ lowered the quantity of heterocentrically organized hippocampal neurons, characterized by a reduction in cell bodies, pycnotic features, and intensely stained nuclei, while the elongated axon became discernible **(Beltagy et al., 2021)**.

In conclusion, this study provides substantial evidence that berberine markedly enhances metabolism, neuroprotection, and antioxidant activity in an animal model of AD induced by D-galactose and $AlCl_3$. Donepezil and berberine enhanced hippocampal histology, neurotransmitter concentrations, metabolic parameters, and oxidative stress indicators. Nevertheless, the synergistic therapy demonstrated more efficacy, suggesting that donepezil and berberine may function collaboratively. The results indicate berberine's potential as an adjunct therapy to enhance the effectiveness of conventional drugs in treating AD. Further clinical study is essential to validate these findings and explore berberine's potential for application in human patients.

Declarations

Ethics approval and consent to participate: The animals were handled and the experiments were done according to the suggested National ethical guidelines for the care of laboratory animals and the Animal Ethics Committee of the Faculty of Science, Tanta Damanhour University (protocol approval code Serial No. (3-12-22).

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Availability of data and material: The data will be available upon request. The data of this article are included within the article and its additional files.

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Authors' contributions:

Eman M. Mostafa: Methodology, validation, writing – original draft

Doha M. Beltagy: Conceptualization, formal analysis, Methodology, supervision, visualization & writing–review & editing.

Tarek M. Mohamed: Conceptualization, formal analysis, Methodology, resources, supervision,

Batoul M. Izzularab: Data curation, investigation, supervision, formal analysis

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memory impairment and increase the expression of interleukin-1beta and inducible nitric oxide synthase in the rat

التأثير العلاجي المفيد للبيربيرين والدونيبيزيل على السمية العصبية في الجرذان.

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٢. **الكيمياء الحيوية - قسم الكيمياء - كلية العلوم - جامعة طنطا**

مرض الزهايمر هو مرض عصبي يتسم بضعف الإدراك، والإجهاد التأكسدي، وتلف الخلايا العصبية. كلوريد البربرين هو مكمل غذائي مضاد للأكسدة يمكن أن يساعد في علاج الإجهاد التأكسدي الناتج عن إصابة الدماغ. هدفت هذه الدراسة إلى التحقق من الإمكانات العلاجية للبربرين، كما هدفت إلى التثبت من أي تأثيرات تآزرية عند اقترانه بعلاج الدونيبيزيل على إصابة الدماغ في الفئران. اجريت الدراسه التجريبيه على سبع مجموعات: مجموعة ضابطه حصلت على محلول ملحي ، ومجموعة ضابطه للبربرين ، ومجموعة ضابطه للدونيبيزيل، مجموعة تم حقنها بكلوريد الالمونيوم و الجالكتوز ، مجموعة معالجة بالبربرين ، مجموعة معالجة بالدونيبيزيل ، ومجموعة معالجة بكلا من البربرين والدونيبيزيل. تم دراسه المؤشرات البيوكيميائية مثل نشاط انزيم الكاتالاز، ومستويات النورأدرينالين، ووظائف الكبد والكلى، ومستويات الدهون، ومستويات الكالسيوم كما أجريت دراسة نسيجية لتقييم سلامة الخلايا العصبية. وقد أظهرت مجموعة نموذج مرض ألزهايمر انخفاضًا كبيرًا في نشاط انزيم الكاتالاز والنورادرينالين والألبومين والكوليسترول الحميد، بالإضافة إلى زيادة كبيرة في إنزيمات الكبد واليوريا والكرياتينين وتركيز الكالسيوم مع تلف خطير في الخلايا العصبية في الحصين.

و قد اثبتت النتائج التي تم الحصول عليها ان البربرين أو الدونيبيزيل قد قاما بمفردهما بتصحيح هذه التغيرات جزئيًا، مما أدى إلى تحسين الدفاع المضاد للأكسدة وتوازن الناقلات العصبية ومورفولوجيا الأنسجة .وقد أسفر العلاج بالبربرين و الدونيبيزيل معا عن استعاده التغيرات الكيميائية الحيوية والمورفولوجية الأكثر أهمية في الحصين بصوره مؤثره احصائيا بما يتجاوز تأثيرات أي من العلاجين بمفردهما. و تلخيصا لهذه النتائج فقد أظهر البربيرين حماية عصبية كبيرة، وكان لتناوله مع الدونيبيزيل تأثيرات تآزرية ضد أمراض شبيهة بمرض الزهايمرمما يوكد أن البربيرين يمكن ان يمثل نهجًا قابلاً للتطبيق في علاج مرض ألزهايمر .