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Original Article

Therapeutic Potential of Plant Based HMG CoA Reductase Inhibitor-Berberine for Alzheimer's Disease in ICV Induced Streptozotocin Rodent Model

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ABSTRACT

Background: Alzheimer's Disease (AD), is a progressive neurodegenerative disease, that results in memory loss, cognitive impairment and behavioral abnormalities. It is one of the most widely spread diseases and is common among elders. In AD there is an increase in the generation of ROS, RNS and phosphorylation of TAU. As a result, efforts to produce AD medications have centered on lowering the levels of Aβ (Amyoid β). Cholesterol stabilizes β-secretase and γ-secretase and promotes amyloidogenesis. **Aim:** The current work evaluated the therapeutic potential of plant-based berberine for Aβ clearance in a rodent model of AD via HMG CoA reductase inhibitory action. **Materials and Methods:** The olfactory sensibility (Buried pellet test), spatial memory (Morris water maze) and recognition memory (Novel Object Recognition Test-NORT) were used to evaluate berberines' protective effects using female C57BL/6 mice. It was reported that Streptozotocin (STZ) causes memory loss and dysregulation of lipid metabolism. **Results:** The current investigation showed encouraging results in slowing the progression of AD by reducing Aβ deposition. The combination of Donepezil and berberine (DPZ-3.5 mg/kg and BBR-100 mg/kg/p.o) had significant improvement in behavioral parameters such as cognitive agility and olfactory function. BBR (100 mg/kg) has shown a significant decrease (40%) in total cholesterol levels compared to the disease group. Additionally, the group treated with BBR (100 mg/kg) alone showed a significant reduction in the Aβ amyloid deposition. **Conclusion:** This study shows that berberine slows the progression of AD by regulating the lipid metabolism as a result reducing the deposition of Aβ amyloid.

Keywords: Alzheimer's disease, Aβ, Donepezil, Berberine, Streptozotocin.

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INTRODUCTION

Alzheimer's Disease (AD) is a neurodegenerative disorder that results in cognitive impairment and may eventually lead to death from complete brain failure. The main pathology of AD is the accumulation of β-amyloid plaques and neurofibrillary tangles by Tau hyperphosphorylation.[1] Alteration in the cleavage of Amyloid Precursor Protein (APP), by β-secretases (BACE1) and γ-secretases results in the formation of insoluble Aβ oligomers, which later develop into β-amyloid plaques and eventually disturb the synaptic functioning. The polymerization of plaques will eventually activate kinases which result in hyperphosphorylation and polymerization of microtubule-associated tau protein into insoluble Neurofibrillary Tangles (NFTs).[2]

The brain synthesizes the cholesterol on its own, as the circulating cholesterol cannot cross the Blood-Brain Barrier (BBB). Cholesterol is an important component as it helps in maintaining the plasticity of neurons and regulates cholesterol synthesis in glial cells. Increased cholesterol activates the β-secretase and γ-secretase. The secretases accelerate the cleavage of Amyloid Precursor Protein (APP) which results in increased aggregation of β-amyloid. From the data collected through various studies, it is revealed that the presence of the ε4 allele of ApoE which is a cholesterol carrier is the hallmark of onset of the disease.[3-5]

Streptozotocin is the most commonly used chemical for the induction of Alzheimer's disease through ICV. It is a cytotoxic compound belonging to the category of Glucosamine nitrosourea.[6] The metabolism of this compounds results in oxidative stress, Deoxyribonucleic acid damage and diabetes mellitus. Streptozotocin inhibits the glucose and glycogen metabolism in the cerebral cortex and hippocampus. This results in the inhibition of insulin receptor function which eventually affects learning, memory and cognitive behavior.

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Insulin resistance causes neuroinflammation. The progression of neuroinflammation results in memory impairment and oxidative stress. This leads to cholesterol dysregulation and the progression of AD.[7]

Various studies show that Berberine vulgaris has shown anti-diabetic and hypoglycemic effects.[8] It enhances glucose metabolism and reduces the damage caused by Streptozotocin.[9] The hypothesis was that berberine which had cholesterol-lowering properties was evaluated for AD. So, the current work focuses on the evaluation of the therapeutic potential of berberine for Aβ clearance using a female C57BL/6 mice model. Behavioral parameters like the buried pellet test, Morris water maze test and Novel object recognition test were performed to evaluate *the* olfactory sensibility and spatial memory. The biochemical parameters like antioxidant, cholesterol levels, Reactive Oxygen Species (ROS) and Acetyl Choline Esterase (AchE) levels were evaluated.

MATERIALS AND METHODS

Treatment drugs

Donepezil was procured from JSS Hospital in Mysuru, Karnataka, India.

Reagents, chemicals/kits

Chemicals like Streptozotocin (STZ) were procured from juniper SRL chemicals (AS-242224). Cholesterol, HDL (High-density Lipoproteins), LDL (Low-density lipoproteins) and TG (Triglycerides) were measured by enzymatic assay from Serum by using commercial AGGAPPE KITS in LABSYSTEMS DIAGNOSTICS biochemical analyzer.

Experimental animals

The experiments on animals were conducted according to the rules of the Committee for Control and Supervision of Experiments on Animals (CCSEA). The protocol was approved by IAEC (NO: JSSAHER/CPT/IAEC/141/2021) and care was taken to reduce the pain of animals. 2-3-month-old female C57BL/6 mice were procured from a CCSEA-approved breeder. Animals were quarantined and provided with sufficient food and water. They are maintained at a 12-hr light/dark cycle under 23°C±2°C temperature. 8 animals were used in each group because ICV surgery might result in complications and mortality. C57BL/6 is used in this study, as they are effective while performing Morris water maze when compared to other strains.[10]

Induction of ad by ICV-stz in mice (surgical procedure)

Anesthetize the mice by intraperitoneal injection of a mixture of ketamine (80-100 mg/kg) and Xylazine (10-12.5 mg/kg) before the ICV injection. The STZ group will receive a single dose of ICV injection of STZ (2.5 µL) by 20 µL of gauge Hamilton syringe. A midline sagittal incision will be made in the scalp. A hole will be drilled through the skull for placement of an infusion cannula into the hippocampus using the following coordinates: AP -0.5 mm; ML ±1.1 mm; DV-2.8 mm relative to the bregma. After surgical and STZ infusion procedures, all animals were kept on a heating pad (37°C) until they had recovered from anesthesia and then maintained in a sterile environment in isolation to minimize the potential infection and mortality. Post-surgical care was taken.[11]

Grouping of animals for induction of ad by mouse model

The animals were randomly divided into 6 groups with 8 animals in each group the grouping was described in Figure 1a and the various evaluation parameters and the duration of the study were described in Table 1. The overall study design is explained in Figure 1b.

Behavioral parameters

Buried Pellet test

The food pellet is buried under the layer of bedding. Animals that take 15 min, to locate the food are considered to have olfactory impairment. On the other hand, animals that locate the food within a few minutes are with normal olfaction. Animals are given 1-2 pieces of pellets before testing for familiarization. This phase is crucial because the mice must become used to the pellet odor. During the test phase, the mice are placed at the center of the cage and the time taken for the mouse to locate the pellet is noted and compared between groups.[12]

Morris water maze

Spatial learning and memory performance were evaluated using the Morris water maze. The pool was filled with water. Standard temperature, opacity and light intensity are maintained. The pool was divided into 4 quadrants. A 5-day acquisition period was adopted, with 2 trials conducted every day for the first 2 days and one trial per day for the final 3 days. The test day was the last day, the sixth. The mice were randomly placed in water and were allowed to swim; the mouse that reached the platform within 60 sec was taken for the study. A 60 sec probe analysis was performed on the last day to evaluate memory preservation. The time spent at the target zone was noted and compared within the groups; a digital camera was used for recording the experiment.[13]

Novel Object Recognition Test

The test was conducted for 5 min with 3 sessions: habituation, training and testing. For 5 min, every animal became accustomed to the empty apparatus. Animals were put within the apparatus on the training day so they could examine 2 familiar objects (object A). Following a 24 hr training period, animals were permitted to explore objects where a new object (B) had replaced one of the

familiar ones. The time spent with the novel object was recorded and compared with other groups.[13]

Biochemical parameters

ROS estimation

2,7- Dichlorodihydrofluorescein Diacetate (DCFH-DA) reagent was used to estimate the ROS levels. Upon reaction with ROS, DCFH-DA gets converted into fluorescent DCF. The DCF levels are analyzed and compared. The brain tissues were collected and homogenized using 0.9% NaCl solution. The solution was centrifuged at 800 rpm for 10 min at 10°C and the supernatant was collected. To the 2 µL supernatant, 198 µL PBS was added. 15 µM DCFH-DA solution was added to 100 µL of prepared solution and the solution was transferred to 96 well microplate. The levels of ROS were detected using a multimode microplate reader at an excitation wavelength of 485 nm and an emission wavelength of 525 nm.[14]

Cholesterol, LDL, HDL, triglycerides

The total cholesterol, triglycerides, HDL and LDL were measured by using the AGGAPPE KITS in LABSYSTEMS DIAGNOSTICS biochemical analyzer.

Antioxidant Estimation

Various antioxidant assays like Catalase assay, Superoxide Dismutase assay (SOD), Reduced Glutathione assay (GSH), Lipid Peroxidation (LPO) and Total protein levels were performed by following the method suggested by Yue Liu *et al.* and Y Lakshmisha Rao *et al.*[15,16]

AchE activity

The isolated brain was homogenized using 0.1 M phosphate buffer (pH 8.0). This homogenate is added to the cuvette which consists of 2.6 mL phosphate buffer (0.1 M, pH 8.0) and 100 µL of DTNB. The absorbance was measured at 412 nm in a KKB spectrophotometer. Acetylthiocholine was added to the twenty microliter of the substrate and the change in absorbance for 10

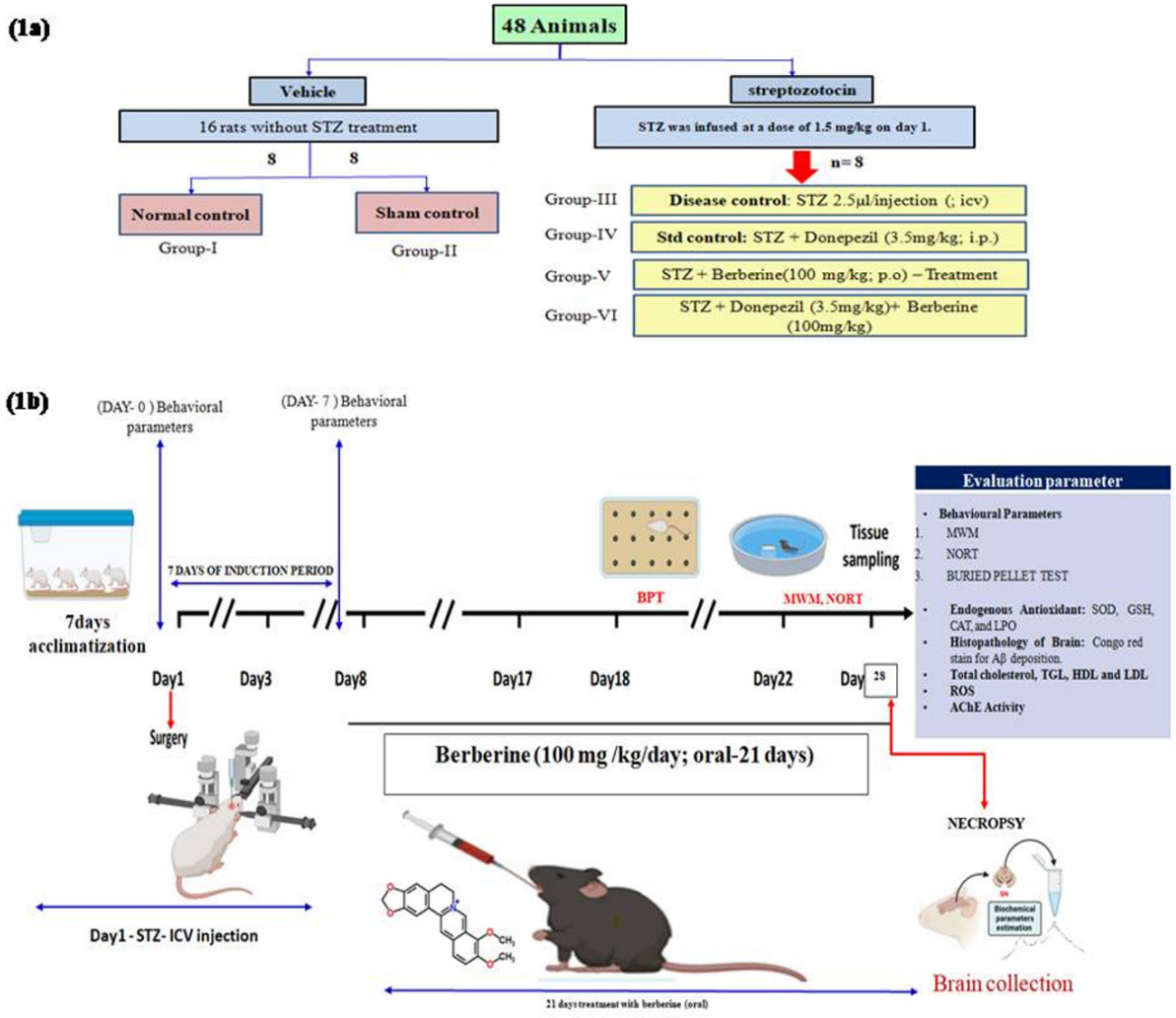

Figure 1: 1a) Grouping of animals, 1b) Overview of the study design.

min at 2 min intervals. The results were expressed as nmol/min/g tissue.[17]

Statistical analysis

Graph pad prism software was used for statistical analysis. The data were represented as mean±SEM. The result was analyzed using two-way Analysis of Variance (ANOVA) and One-way Analysis of Variance (ANOVA) followed by Tuckey's multiple comparison test. The value of $p<0.05$ was considered statistically significant.

RESULTS

Effect of berberine on olfactory function after STZ induction in mice

Sham control did not show any significant changes in the time taken to unbury the buried pellet from day 0 to day 35 (8.83±0.70 vs. 13.16±1.013). The disease group had shown increased time to unbury the pellet from day 0 to day 35 (8.5±0.428 vs. 34±1.570) when compared to normal. Berberine-treated groups have shown an increased time taken to unbury the buried pellet on day 28 when compared to standard (23.83±1.222 vs19.83±1.30). A combination of (berberine and Donepezil) has shown an increase in the time taken to unbury the buried pellet on day 28 when compared to standard (22.33±0.843 and 19.833±1.301). A combination of (berberine and donepezil) has a significant decrease in the time taken to unbury the buried pellet on day 28 when compared to the disease group (34±1.570 vs. 22.33±0.843). The effect of berberine on the buried pellet test was reported in Figure 2a.

Effect of berberine on memory function in mice after STZ induction in mice

Effect of Berberine on Time Spent in target quadrant after induction in Mice

Sham control did not show any significant changes in the time spent in the target quadrant from day 0 to day 28 (15.21±2.05 vs. 15.65±1.00). The disease group showed a decrease in the time spent in the target quadrant from day 0 to day 28 (17.32±0.51 vs 10.61±1.62) when compared to normal. Berberine-treated groups have not shown any significant variation in time spent in the target quadrant on day 28 when compared to standard (13.61±0.92 vs. 13.77±0.97). A combination of (DPZ and BBR) has a slight difference in a variation on day 28 when compared to standard (17.15±0.7vs 13.77±0.97).The effect of berberine on time spent in the target quadrant was reported in Figure 2 b.

Effect of Berberine on latency to reach target quadrant after Aβ induction in mice

Sham control did not show any significant changes in latency to reach the target quadrant from the day on day 28 when compared to normal (13.14±1.14 vs. 9.75±0.40). The disease group showed latency to reach the target quadrant from day 28 (17.72±1.20 vs. 9.18±1.3) when compared to normal. Berberine-treated groups have shown a significant increase in latency to reach the target quadrant day 28 when compared to standard (10.02±1.15

Table 1: Grouping of animal, dosing and duration of study.

Groups	No. of animals	Treatment dose, duration and route	Evaluation parameters
Group 1 Normal	8	Vehicle - Saline	Behavioral parameters: Morris water maze Novel object recognition test Buried pellet Test Biochemical Parameters: Totalcholesterol- HDL, LDL and TGL. ROS estimation Antioxidant's estimation AchE activity.
Group 2 Sham control	8	Sham control-saline	
Group 3 Disease	8	STZ was infused unilaterally at a dose of 1.5 mg/kg/day on day 1 in saline 2.5 µL/injection. Induction period-7 days (ICV).	
Group 4 Standard	8	STZ was infused unilaterally at a dose of 1.5 mg/kg/day on day 1 in saline 2.5 µL/injection. Induction period -7 days (ICV)+donepezil (3.5 mg/kg; p.o) 21 days of study.	
Group 5 Treatment	8	STZ was infused unilaterally at a dose of 1.5 mg/kg/day on day 1 in saline 2.5 µL/injection+Berberine (100 mg/kg; p.o) (28 days of study).	
Group 6 combination	8	STZ was infused unilaterally at a dose of 1.5 mg/kg/day on day 1 in saline 2.5 µL/injection. Induction period-7 days (ICV)+Berberine (100 mg/kg)+donepezil (3.5 mg/kg) 21 days of study.	
Total number of animals	48		

vs. 8.54±0.62). A combination of (Berberine and DPZ) has a significant difference in latency to reach target quadrant variation on day 28 when compared to standard (9.11±0.55 vs. 8.54±0.62). The effect of berberine on latency to reach the target quadrant was reported in Figure 2c.

Novel object recognition test

Effect of Berberine on Time spent near novel object after STZ induction of AD in mice

Sham control animals did not show any significant changes in the time spent near novel objects from day 0 to day 28 (4.8±0.7 vs. 4.1±0.6). Disease control showed a decrease in time spent near novel objects from day 7 to day 28 (1.4±0.7 vs. 1.5±0.5) when compared to normal control. On day 7 all the treated groups showed a significant decrease in the time spent near novel objects when compared to sham control (1.4±0.7, 1.0±0.3, 1.3±0.2, 1.6±0.4, 1.5±1.1 vs. 2.4±0.3). On day 28 there was a significant increase in time spent near novel objects when compared to disease control (1.5±0.5, 3.4±0.5, 3.1±0.7, 3.0±0.41 vs. 4.1±0.6). On day 28 among the groups, standard, treatment and combination showed a significant increase in time spent near novel objects when compared to disease control (3.4±0.5, 3.1±0.7, 3.0±0.41 vs. 1.5±0.5). The effect of berberine on Time spent near novel objects was reported in Figure 2d.

Discrimination index: Effect of Berberine on % Discrimination index after STZ induction in mice

The discrimination index of sham control animals has varied non-significantly from day 0 to day 28 (29.75±3.4, 20.06±0.9, 40.60±5.4). Discrimination index of disease control has decreased significantly from day 7 to day 28 (10.35±1.5, 5.77±1.9). On day 7 all the treated groups showed a non-significant decrease in the Discrimination index when compared to disease control (15.43±2.7, 11.26±2.5 vs. 10.35±1.5). On day 28 there was a significant increase in the Discrimination index when compared to disease control (57.71±3.9, 50.24±5.8, 39.63±3.6 vs. 5.77±1.9). On day 28 among the group's standard, treatment and combination showed a significant increase in the Discrimination index when compared to disease control (57.71±3.9, 50.24±5.8, 44.63±3.6vs. 5.77±1.9). The effect of berberine on the % Discrimination index was reported in Figure 2e.

Effect of berberine on ROS level after STZ induction of ad in mice

The Sham group did not show any significant increase in the ROS when compared to normal (100±12 vs 88±10.0). The disease group has shown an increase in the amount of ROS when compared to normal (175.10±15.0 vs. 88±10.0). Donepezil treated group has shown a significant decrease in the level of ROS when compared to the disease (125.64±6.00 vs. 175.10±15.0). Berberine treated group has shown a significant decrease in the level of ROS when compared to the disease (137.79±9.00 vs. 175.10±15.0). A combination of (DPZ and BBR) has shown a decrease in the amount of ROS when compared to the disease group (112.27±5.00vs. 175.10±15.0). The effect of berberine on Total ROS levels was reported in Figure 3.

Lipid profile

Effect of berberine on Total cholesterol after STZ induction in mice

The Sham group did not show any significant increase in the total cholesterol when compared to normal (38.92±2.37 vs 40.31±2.73). The disease group showed an increase in the amount of total cholesterol when compared to normal (59.89±0.95 vs. 40.31±2.73). Berberine treated group has shown a decrease in the amount of total cholesterol when compared to the disease (37.68±2.48 vs. 59.89±0.95). A combination of (DPZ and BBR) treated groups has shown a decrease in the amount of total cholesterol when compared to disease (41.07±1.91 vs. 59.89±0.95). The effect of berberine on Total cholesterol was reported in Figure 4a.

Effect of Berberine on Triglycerides after STZ induction in mice

The Sham group did not show any significant increase in the triglycerides when compared to normal (48.73±2.30 vs 50.91±1.99). The disease group showed an increase in a number of triglycerides when compared to normal (64.10±2.33 vs. 50.91±1.99). Berberine treated group has shown a decrease in the amount of total cholesterol when compared to the disease (51.91±1.99 vs. 64.10±2.33). A combination of (DPZ and BBR) treated groups has shown a decrease in the amount of triglycerides when compared to disease (49.33±2.80 vs. 64.10±2.33). The effect of berberine on triglycerides was reported in Figure 4 b.

Effect of Berberine on HDL after STZ induction in mice

The Sham group did not show any significant increase in the HDL when compared to normal (28.80±3.48 vs. 27.81±2.66). The disease group showed an increase in the amount of HDL when compared to normal (41.40±1.06 vs. 27.81±2.66). Donepezil treated group has shown a decrease in the amount of HDL when compared to the disease (39.59±0.96 vs. 41.40±1.06). Berberine treated group has shown a significant decrease in the amount of HDL when compared to the disease (40.44±1.00 vs.41.40±1.06). A combination of (DPZ and BBR) has shown a decrease in the amount of HDL when compared to the disease group (33.52±1.98 vs. 41.40±1.06). The effect of berberine on HDL was reported in Figure 4c.

Effect of Berberine on LDL after STZ induction in mice

The Sham group did not show any significant increase in the LDL when compared to normal (18.4±1.53vs 17±2.66). The disease group showed an increase in the amount of LDL when compared to normal (38.6±3.66 vs. 17±2.66). Donepezil treated group has shown a decrease in the amount of LDL when compared to the disease (25.75±0.96 vs. 38.6±3.66). Berberine treated group has shown a significant decrease in the amount of LDL when compared to the disease (21.4±1.00 vs. 38.6±3.66). A combination of (DPZ and BBR) has shown a decrease in the amount of LDL when compared to the disease group (19.4±1.66 vs. 38.6±3.66). The effect of berberine on LDL was reported in Figure 4d.

Antioxidant levels estimation

Effect of Berberine on GSH levels after STZ induction of AD in mice

The Sham group did not show any significant difference in the GSH activity when compared to normal (62.6±3.95% vs. 58.21±3.55%). The disease group showed a significant decrease in the amount of GSH when compared to normal (31.53±1.74% vs. 62.6±3.95%). The standard treated group has shown an increase in the level of GSH when compared to the disease (49.31±2.17% vs. 31.53±1.74%). Berberine treated group has shown a significant increase in the level of GSH when compared to the disease (43.90±1.81% vs. 31.53±1.74%). A combination of (DPZ and BBR) has shown an increase in levels of GSH when compared to the disease group (50.60±2.94% vs. 31.53±1.74%). The effect of berberine on GSH levels was reported in Figure 5a.

Effect of Berberine on SOD levels after STZ induction of AD in mice

The Sham group did not show any significant difference in the SOD level when compared to normal (36.21±2.17% vs. 39.61±2.11%). The disease group showed a significant decrease in the amount of SOD when compared to normal (18.27±2.59% vs. 39.61±2.11%). The standard treated group has shown an increase in the level of SOD when compared to disease (31.53±1.74% vs. 18.27±2.59%). Berberine treated group has shown a significant increase in the level of SOD when compared to the disease (26.87±1.85% vs. 18.27±2.59%). A combination dose of (DPZ and BBR) has shown an increase in levels of SOD when compared to the disease group (29.90±2.86%vs. 18.27±2.59%). The effect of berberine on SOD was reported in Figure 5b.

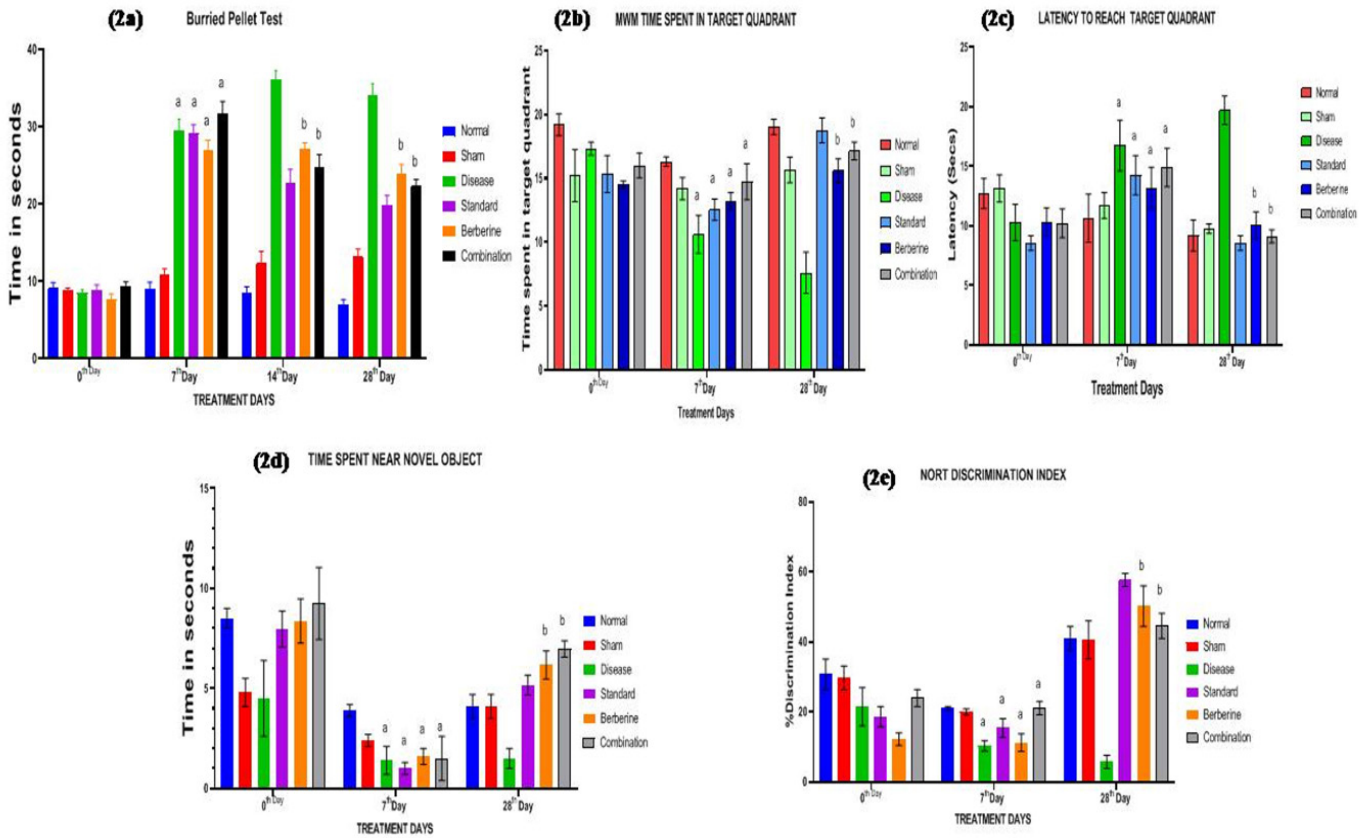

Figure 2: Effect of berberine on 2a) olfactory function after STZ induction in mice 2b) Time spent in target quadrant after STZ induction in mice 2c) latency to reach target quadrant after Aβ induction in mice 2d) Time spent near novel object after STZ induction of AD in mice 2e) % Discrimination index after STZ induction in mice. All values are expressed in the form of Mean±SEM, *n*=8, data were analyzed by employing Two-way ANOVA and 'a' represents *p*-value<0.05 when compared to Normal vs. disease and 'b' represents *p*-value<0.05 when compared to disease vs. treatment groups.

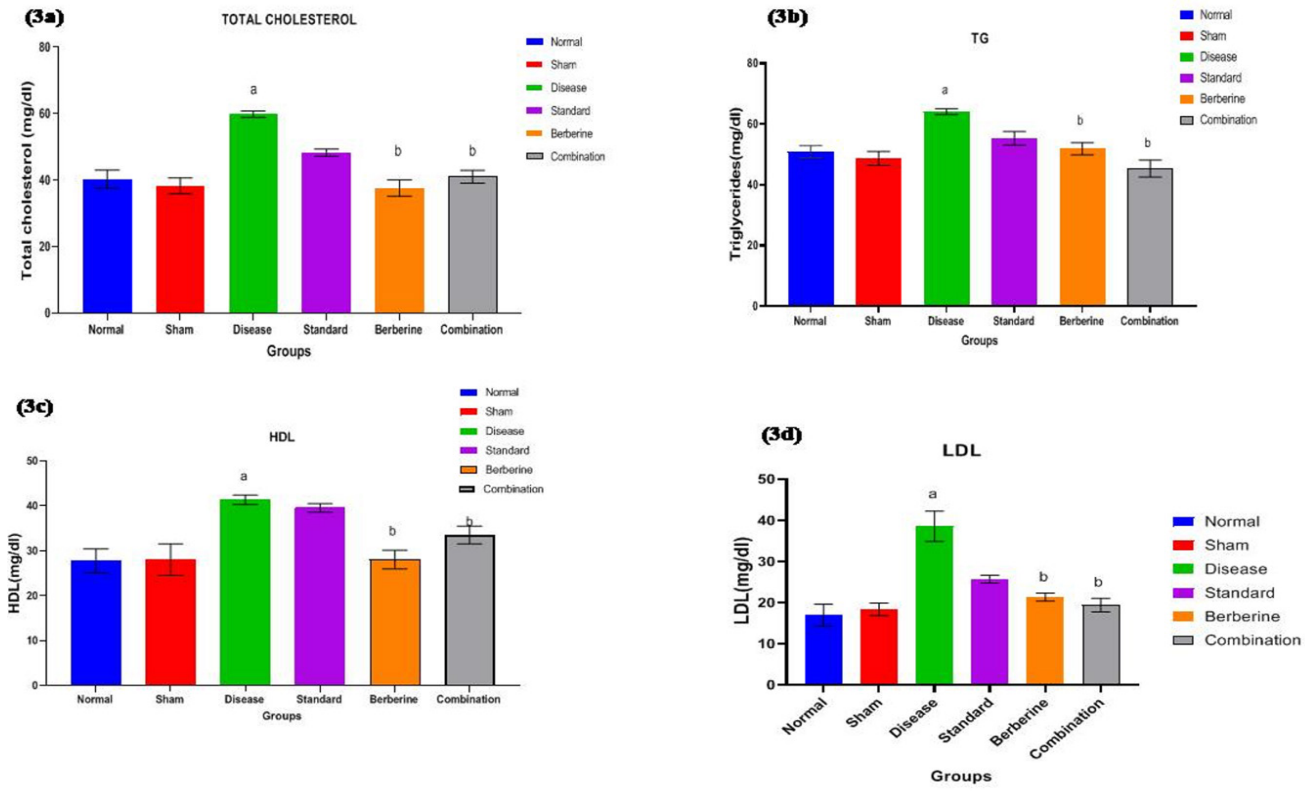

Figure 3: Effect of berberine on 3a) Total cholesterol after STZ induction in mice 3b) Triglycerides after STZ induction in mice 3c) HDL after STZ induction in mice 3d) LDL after STZ induction in mice. All values are expressed in the form of Mean±SEM, *n*=8, data were analyzed by employing Two-way ANOVA and 'a' represents *p*-value<0.05 when compared to Normal vs. disease and 'b' represents *p*-value<0.05 when compared to disease vs. treatment groups.

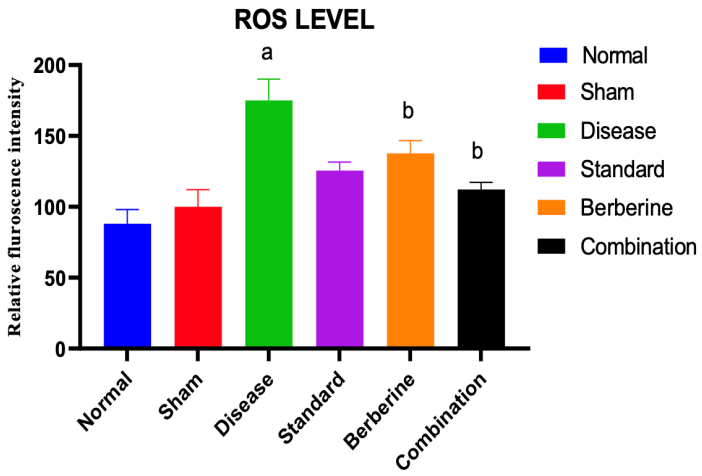

Figure 4: Effect of Berberine on ROS level after STZ induction of AD in mice. All values are expressed in the form of Mean±SEM, *n*=8, data were analyzed by employing Two-way ANOVA and 'a' represents *p*-value <0.05 when compared to Normal vs. disease and 'b' represents *p*-value < 0.05 when compared to disease vs. treatment groups.

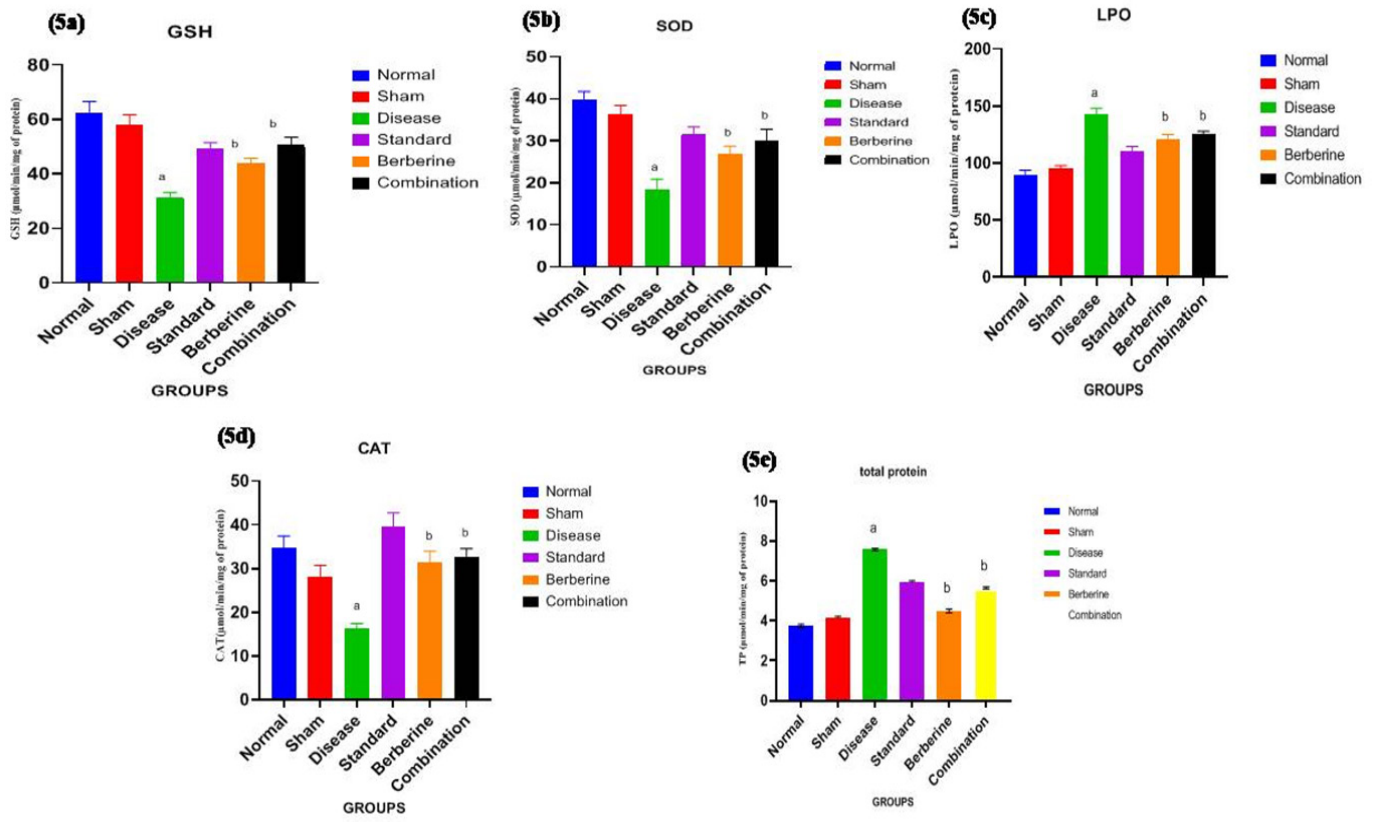

Figure 5: Effect of Berberine on a) GSH levels after STZ induction of AD in mice b) SOD levels after STZ induction of AD in mice c) LPO levels after STZ induction of AD in mice d) CAT levels after STZ induction of AD in mice e) Total Proteins levels after STZ induction of AD in mice. All values are expressed in the form of Mean±SEM, *n*=8, data were analyzed by employing Two-way ANOVA and 'a' represents *p*-value<0.05 when compared to Normal vs. disease and 'b' represents *p*-value<0.05 when compared to disease vs. treatment groups.

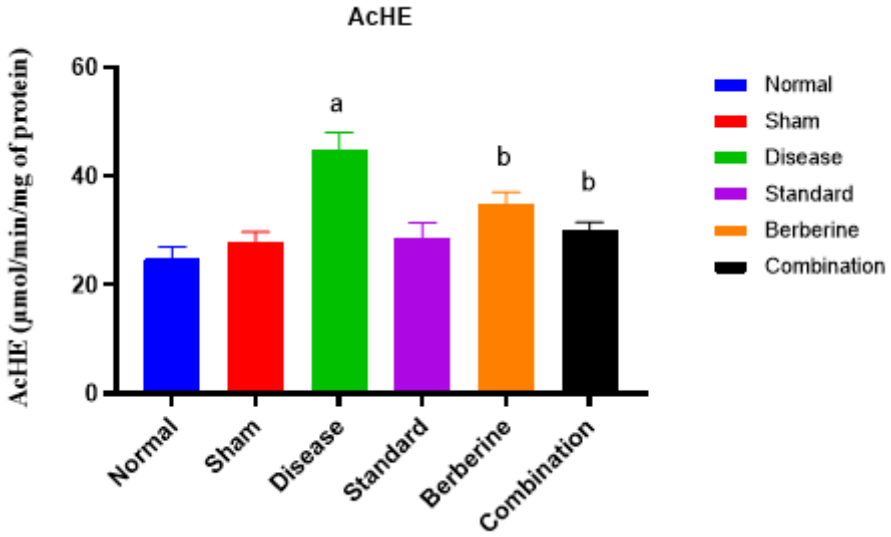

Figure 6: Effect of Berberine on AchE activity after STZ induction of AD in mice. All values are expressed in the form of Mean±SEM, *n*=8, data were analyzed by employing Two-way ANOVA and 'a' represents *p*-value<0.05 when compared to Normal vs. disease and 'b' represents *p*-value<0.05 when compared to disease vs. treatment groups.

Effect of Berberine on LPO levels after STZ induction of AD in mice

The Sham group did not show any significant difference in the LPO activity when compared to normal (99.31±3.57% vs. 95.81±3.84%). The disease group showed a significant increase in the amount of LPO when compared to normal (142.90±4.70% vs. 89.81±3.84%). The standard treated group has shown a decrease in the level of LPO when compared to the disease (115.61±3.84% vs. 142.90±4.70%). Berberine treated group has shown a significant decrease in the level of LPO when compared to the disease (128.17±3.74% vs. 142.90±4.70%). A combination of (DPZ and BBR) has shown a decrease in levels of LPO when compared to the disease group (122.87±6.18%vs. 142.90±4.70%). The effect of berberine on LPO levels was reported in Figure 5c.

Effect of Berberine on CAT levels after STZ induction of AD in mice

The Sham group did not show any significant difference in the CAT activity when compared to normal (31.44±2.58% vs. 34.66±2.78%). The disease group showed a significant decrease in the amount of CAT when compared to normal (16.33±1.07%v s. 34.66±2.78%). the standard treated group has shown an increase in the activity of CAT when compared to the disease (43.66±3.05% vs. 16.33±1.07%). Berberine treated group has shown a significant increase in the level of CAT when compared to the disease (25.66±2.51% vs. 16.33±1.07%). A combination of (DPZ and BBR) has shown an increase in levels of GSH when compared to the disease group (32.76±1.78% vs. 16.33±1.07%). The effect of berberine on CAT levels was reported in Figure 5d.

Effect of Berberine on Total protein levels after STZ induction of AD in mice

The Sham group did not show any significant difference in the TP level when compared to normal (4.16±0.08% vs. 3.74±0.08%). The disease group showed a significant increase in the amount of TP when compared to normal (7.57±0.06% vs. 3.74±0.08%). The standard treated group has shown a decrease in the level of TP when compared to the disease (5.94±0.24%vs. 7.57±0.06%). Berberine treated group has shown a significant decrease in the level of TP when compared to the disease (4.50±0.09% vs. 7.57±0.06%). A combination of (DPZ and BBR) has shown a significant decrease in levels of GSH when compared to the disease group (5.64±0.05%vs. 7.57±0.06%). The effect of berberine on Total protein levels was reported in Figure 5e.

Effect of berberine on AchE activity in STZ induced mice

The Sham group did not show any significant increase in the AchE activity when compared to normal (27.87±1.87% vs. 24.90±2.14%). The disease group showed an increase in the amount of AchE when compared to normal (44.91±3.13% vs. 24.90±2.14%). Donepezil treated group has shown a significant decrease in the level of AchE when compared to the disease (28.57±2.90% vs. 44.91±3.13%). Berberine treated group has shown a significant decrease in the level of AchE when compared to the disease (34.93±2.09% vs. 44.91±3.13%). A combination of (DPZ and BBR) has shown a decrease in the amount of AchE when compared to the disease group (30.25±1.25%vs. 44.91±3.13%). The effect of berberine on AchE was reported in Figure 6.

DISCUSSION

AD is a terminal illness caused by hereditary and environmental factors. Despite recent promising developments in the study of AD, the link between cholesterol and APP processing remains poorly understood. During AD condition the cholesterol levels in the brain increase and these regions are called lipid rafts. These lipid rafts are hosts for β and γ secretases. As a result, they develop mono fibrils, which lead to oligomerization of $A\beta_{1-42}$ and, eventually accumulation of Aβ. Cholesterol inhibits the α-secretase and non-amyloid pathways as well. Berberine shows its effectiveness by reducing cholesterol from CSF which will eventually reduce the amount of Aβ peptides. A reduction in the amount of Aβ peptides indicates recovery from disease. Donepezil exerts its effect by reversibly inhibiting the AchE and is capable of crossing BBB. In the present study, administration of ICV-STZ by a single unilaterally method was used for induction of AD in rodents. After ICV surgery, the female C57BL/6 mice showed AD symptoms like olfactory damage and cognitive impairment. The cognitive impairment was developed upon Streptozotocin administration, by modulating the glucose and energy metabolism of the brain. The decline in the levels of Ach might be the reason for olfactory damage. The olfactory damage was assessed by the time taken to unbury the pellet. The cognitive impairment was assessed by the water maze by assessing the time spent in the target quadrant and the latency to reach the target quadrant. In this study administration of ICV-STZ resulted in a decrease in the olfactory function in disease when compared to the normal and sham control. The standard group and the treatment groups have shown a significant increase in olfactory function when compared to the disease control group. Donepezil raises the levels of Ach by inhibiting the enzyme AchE. The raised levels of Ach recover the olfactory damage caused by STZ. The cognitive impairment was assessed by the MWM. The time spent in the quadrant zone was decreased in the disease group and increased in treatment, standard and combination groups when compared to the normal and sham control. Similarly, the latency to reach the target quadrant was also assessed by the Morris water maze. The latency to reach the target quadrant in the disease group was increased and decreased in standard, treatment and combination groups when compared to the normal and sham control. The increase in the time spent in the quadrant zone and decrease in latency to reach the target quadrant, predicts that the drug berberine and Donepezil combination was effective as they were capable of exerting anti-amyloidogenic, anti-AchE

and cholesterol-lowering activity. Similarly, in the NORT study disease induced animals showed reduced preference towards novel objects as evidenced by a decrease in the time spent near novel objects when compared to sham control. After the induction period, the diseased mice were observed to be exploring less time near the novel object. Animals treated with berberine significantly spent more time near the novel object compared to disease control. Mice with combination dose of (BBR and DPZ) have also shown a significant improvement in cognitive impairment. Total cholesterol and triglycerides, HDL and LDL were estimated in serum and were measured by an enzymatic assay using commercial AGAPPE KITS. The amount of total cholesterol and triglycerides, HDL and LDL levels was increased in the disease group compared to the normal. The standard drug-treated group does not have any significant effect on the total cholesterol and triglycerides, HDL and LDL. As the standard drug was not proven to have cholesterol-lowering activity. The treatment group's berberine and combination groups have shown a significant decrease in the amount of total cholesterol, triglycerides, HDL and LDL. Antioxidant - GSH, SOD, LPO, CAT and TP levels were estimated in the brain hippocampal region. It was estimated that the levels of GSH, SOD and CAT were significantly decreased in the ICV-STZ group when compared to the normal group. After treatment with the BBR (100 mg/kg/oral) has shown a significant increase in the levels of GSH, SOD as well as CAT. The GSH, SOD and CAT levels were raised upon administration of berberine, Donepezil and a combination drug, indicating the drugs were having antioxidant activity, which will eventually result in anti-inflammatory activity. LPO and TP were also estimated in the brain hippocampal region. It is revealed that the levels of LPO were significantly increased in the ICV -STZ group when compared to the normal group. The treatment with the BBR (100 mg/kg/oral) has shown a significant decrease in the levels of LPO and TP. The levels of ROS were significantly decreased upon administration of Donepezil, berberine and combination drugs. The combination was effective against ROS when compared to Donepezil and Berberine alone. Donepezil which was an established AchE inhibitor, showed a significant decrease in AchE levels compared to berberine and combination. These results show that BBR exhibits cholesterol lowering property by blocking the HMG CoA reductase pathway thereby regulating cholesterol and reducing the deposition of Aβ plaques. And (BBR and DPZ) have a good effect on the improvement of behavioral parameters in AD mice. Hence combination of (BBR and DPZ) has anti Alzheimer's effect by regulating the cholesterol and clearance of Aβ1-42 aggregation by blocking the HMG coA reductase pathway.

CONCLUSION

The results show that the administration of STZ through ICV induces Alzheimer's disease in rodents. Upregulation of lipid profile and decrease in levels of GSH, SOD and CAT levels as well as increase in LPO, TP and beta-amyloid levels are seen in disease-induced animals. Administration of BBR and DPZ significantly improved the olfactory function, cognitive function and the levels of GSH, SOD and CAT levels and decreased the level of LPO and TP. Administration of BBR reduces the total cholesterol, triglycerides, HDL and LDL levels. The levels of ROS and AchE were reduced upon administration of the combination. Hence the study shows that the combination of BBR and DPZ possesses an anti-Alzheimer's effect in STZ-induced Alzheimer's rodent model.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The experiment involves animals which were approved by IAEC (IAEC NO: JSSAHER/CPT/IAEC/141/2021).

ABBREVIATIONS

AD: Alzheimer's disease; **Aβ:** Amyloid β; **ROS:** Reactive oxygen species; **RNS:** Reactive nitrogen species; **STZ:** Streptozotocin; **DPZ:** Donepezil; **BBR:** Berberine; **NORT:** Novel object recognition test; **MWM:** Morris water maze; **APP:** Amyloid precursor protein; **AchE:** Acetylcholine Esterase; **HDL:** High density Lipoproteins; **LDL:** Low density lipoproteins; **TG:** Triglycerides; **GSH:** Glutathione; **SOD:** Superoxide dismutase; **LPO:** Lipid peroxidation; **CAT:** Catalase.

SUMMARY

The study aims to evaluate the anti-Alzheimer's effect of berberine and the combination of berberine and Donepezil. To evaluate the spatial and learning memory, MWM and NORT were performed respectively. To evaluate the olfactory ability Burried pellet test was performed. Cholesterol levels and antioxidant levels were analyzed. All the results suggested that the berberine reduced the cholesterol levels and improved the anti-Alzheimer effect. The BBR reduced the levels of total proteins indicating the anti-amyloidogenic activity. The level of AchE was decreased by BBR indicting BBR can increase the levels of Ach, which was necessary for anti- Alzheimer's activity. From the above findings, berberine might have therapeutic potential against Alzheimer's disease by regulating the cholesterol levels and AchE levels.

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