International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.22.33

Research Article
Improvement of Learning and Memory by Chickpeas (*Cicer arietinum* L.) Sprout Against Streptozotocin and Induced Amnesia in Rats

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Abstract

Background and Objective: Alzheimer's disease (AD), which affects the brain and causes neurodegeneration, has been found to impact insulin signaling pathways related to cell survival, such as PI3/Akt and GSK-3. Previous studies have shown that streptozotocin (STZ) can increase the Bax/ratio, leading to caspase 3 activation and apoptosis. This research aims to investigate whether an extract from chickpeas (CPE) can aid in the recovery of rats from STZ-induced memory loss. **Materials and Methods:** The study involved adult male Sprague Dawley rats weighing between 200 and 250 g. Cannulas were inserted into the ventricles of the rats and they were administered STZ (3 mg kg^{-1}) on days 1 and 3. Starting from day 4 until day 14, CPE treatment at doses of 100 and 200 mg kg^{-1} was given every day. From days 15 to 18, the rats' learning and memory abilities were evaluated using a morris water maze test and a closing field operation. **Results:** On day 21, the rats were sacrificed to measure pathways related to lipid peroxidation (LPO), including malondialdehyde (MDA), Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and acetylcholinesterase (AChE). The rats given CPE showed a dose-dependent improvement in escape latency and step-through latency, along with a decrease in transfer latency. Specifically, the rats treated with 200 mg kg^{-1} of CPE showed improvements in memory-related parameters such as MDA and AChE levels well as increased levels of glutathione and catalase. The CPE at 200 mg kg^{-1} reversed memory loss caused by STZ, whereas a lower dosage of 100 mg kg^{-1} did not. **Conclusion:** The STZ elevated caspase 3 levels and disrupted Akt/GSK 3 signaling in the hippocampus. The CPE at 200 mg kg^{-1} prevented STZ-induced apoptosis and dysfunction of Akt/GSK 3 signaling pathways.

Key words: Alzheimer's disease, Akt/GSK-3 signaling, chickpea, streptozotocin, amnesia, Bax levels

Citation: Jawaid, T., 2024. Improvement of learning and memory by chickpeas (*Cicer arietinum* L.) sprout against streptozotocin and induced amnesia in rats. Int. J. Pharmacol., 20: 22-33.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alzheimer's disease (AD), is a chronic illness that results in gradual cognitive decline and impairs memory, speech and problem-solving skills in those affected. It is a common affliction among the elderly, affecting the vast majority of individuals over the age of 65[1]. The debilitating nature of the disease often requires those affected to seek assistance in their daily lives. According to projections, the prevalence of Alzheimer's disease is expected to increase significantly by 2050, affecting approximately one in 85 individuals worldwide[2]. Further describe the cognitive impairment associated with AD as a global loss of recollection, orientation, thinking and reasoning abilities[3].

There is a general consensus within the scientific community that Alzheimer's disease (AD), which is induced by hippocampal damage, results in episodic memory deficits, delayed recall deficits and impairments in collective experience[4]. However, there is still some debate regarding the patterns of protection and disability in various cognitive skills, such as semantic memory, understanding, slow recognition and working memory, including imagination[5]. Despite these ongoing discussions, the accumulation of the protein fragment beta-amyloid (plaques) and twisted fibers of the tau protein (tangles) within nerve cells is a fundamental characteristic of AD pathogenesis[6]. Beta-amyloid plaques act as neurotoxins by disrupting neuron-to-neuron communication at synapses. Conversely, tau tangles obstruct essential molecules and nutrients from crossing the neurons, leading to signal transduction failure and cell death[7].

Streptozotocin (STZ) ICV is a well-known memory disorder template used to diagnose Alzheimer's disease in rodents[8]. The STZ is a glucosamine nitrosourea derivative first identified as a potential antibiotic. This substance is harmful to pancreatic β-cells and is commonly utilized to induce diabetes in rats in clinical studies. The TZ administration (ICV) results in a decrease in comprehension and an improvement in cerebral consolidated amyloid protein and the production of neurofibril tangles, which is related to memory problems[9]. The CREB/BDNF pathway, activated by the central cholinergic system[10], promotes hippocampal neurogenesis in healthy individuals through increased acetylcholine availability via AChE inhibitors such as tacrine or donepezil[11].

In addition, oxidative stress appears to be involved in Alzheimer's disease, with research linking beta-amyloid toxicity to either a spike in reactive oxygen species (ROS), including H_2O_2[12] or lipid peroxidation throughout neuronal cultures. Memory deficits may be affected by massive oxidative stress, leading to diminished hippocampal synaptic plasticity and oxidative damage in neurodegenerative disorders[13]. As a result, almost all medications in the market aim to enhance memory by inhibiting AChE. Additionally, AD is a multifactorial condition, never caused by a single factor, including AChE and this should be taken into consideration when producing a medication. Other factors leading to psychotic symptoms in AD include oxidative stress and synaptic dysfunction[14].

Natural products may have been a source of neuroprotective drugs because they can maintain regular cellular interactions in the brain and increase the risk of neuronal dysfunction under pathological conditions[15]. Many Alzheimer's drug discovery organizations have investigated natural products as neuroprotective agents. Chickpeas (*Cicer arietinum* Linn.) were the legumes most frequently eaten. It is a foodstuff found in tropical and subtropical areas[16]. Chickpeas are inexpensive and high-quality proteins. It is primarily a dietary ingredient, although it is also a rich source of vitamins, carbohydrates and trace minerals. It is also used for antipyretic, antidiarrheal, antifungal, antibacterial, anti-inflammatory, cold cough and other medicinal purposes[17]. The hydroalcoholic extract of chickpea seeds exhibits anti-oxidant, antidiarrheal and antipyretic properties[18]. The anti-amnesic potential of chickpea seeds on memory deficits in a rat model of streptozotocin-induced cognitive impairment was investigated in the current research.

MATERIALS AND METHODS

Study area: The study was carried out at Hygia Institute of Pharmaceutical Education and Research, Lucknow, India from March, 2018-2019.

Streptozotocin, chloral hydrate, Sodium Chloride (NaCl), 2-thiobarbituric acid (TBA), 1,1,3,3 tetra ethoxy propane (TEP), Potassium Chloride (KCl), Magnesium Chloride ($MgCl_2$), Calcium Chloride ($CaCl_2$), bovine serum albumin (BSA), acetylthiocholine iodide (AChI) and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma Aldrich, Mumbai, India.

Preparation of extract: A modified process was used to produce chickpea sprout extract (CPE)[19]. Seeds from three varieties (PBG-1, GPF-2 and PBG-5) were gathered and washed with clean water. The seeds were spread on a wet cotton mat and covered with a similar type of cotton cover. Water was sprinkled as needed to keep the mat moist. Sprouts with a germination length of 1.5-3.0 cm were chosen for the study and it took approximately 72 hrs for the seeds to reach this stage of germination. A crystalline substance was prepared

Table 1: GC-MS data of chickpeas determines peak area and retention time of pharmacologically active compounds

Peak	R. Time	Area	Area (%)	Name
1	6.291	1545662	1.12	4-Hydroxy-3-methyl-2-butenyl acetate
2	9.327	33235981	24.04	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
3	11.168	19624348	14.19	Ethyl. alpha. -d-glucopyranoside
4	12.305	45974854	33.25	Mome inositol
5	13.641	274070	0.20	9,17-Octadecadienal, (Z)-
6	15.342	2180822	1.58	Hexadecanoic acid, ethyl ester
7	19.667	14066760	10.17	Ethyl (9Z,12Z)-9,12-octadecadienoate #
8	19.880	3884082	2.81	Ethyl Oleate
9	20.766	459122	0.33	Hexadecanoic acid, Ethyl ester
10	23.309	1432340	1.04	1-Hydroxy-2,2,6,6-Tetramethyl-3-(1-piperidin
11	23.424	249952	0.18	1-Hydroxy-2,2,6,6-Tetramethyl-3-(1-piperidin
12	23.823	168422	0.12	Hexadecadienoic acid, Methyl ester
13	25.554	224796	0.16	(6E,11Z)-1,6,11-Hexadecatriene #
14	25.620	210700	0.15	2-Hydroxy-3-[(9E)-9-octadecenoyloxy] Propy
15	28.781	12182577	8.81	9-Octadecenoic acid (Z)-
16	34.957	412635	0.30	Ergost-5-en-3-ol, (3. Beta.,24R)-
17	35.462	351125	0.25	Stigmasterol
18	36.836	1464894	1.06	Stigmast-5-en-3-ol, (3. Beta, 24S)-
19	37.753	336489	0.24	Olean-12-en-3-ol, acetate, (3 beta)-
		138279631	100.00	

from the raw sprouts and the powders were soaked in 100% methanol for three days. Vacuum filtration was used to reduce the volume of the supernatant to 1/3 and the sample was derivatized with trimethylsilyl (TMS) for GC-MS analysis. The Xcalibur software was used to analyze the GC-MS results and the chromatogram of the GC-MS profile was analyzed using the WILLY and NIST mass spectral libraries[20].

GC-MS analysis: The extract of n-hexane of pumpkin seed oil was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis using a Shimadzu QP-2010 Ultra instrument, which was equipped with a capillary non-polar 60 M TRX-5 MS column. Helium gas was carrier gas and flowed at 1.21 mL min^{-1}. The instrument temperature gradually increased from 100 to 260°C at 10°C per minute and the injection volume was set at 2 µL. For the GC-MS analysis, electron ionization with an ionization energy of 70 eV was utilized. The sample was dissolved using n-hexane as the solvent and then scanned within the mass between 10 and 870 m/z for 30 min. The obtained data were compared and analyzed using Wiley library software. Mass spectra were identified within the 0-30 min time frame. Compound names, molecular weights, formulas and structures were determined and the relative abundance of each compound was assessed by comparing its peak area to the total peak areas. Furthermore, the identity of each compound was confirmed by comparing retention times and spectra with reference standards (Sigma-Aldrich) and NIST Mass Spectral Library Version 2.0 d mass spectra.

In vivo studies

Animals: The 18 Sprague Dawley (SD) rats were sourced from a national breeding center and were kept in conventional circumstances in polyacrylic cages with a 12 hrs light/dark cycle, a temperature range of 22-26°C, a humidity of 50-70% and a constant supply of food. The study was approved by the Institutional Animal Ethics Committee (IAEC) and complied with the standards set by CPCSEA (HIPER/1088/07).

Treatment schedule: For the experiment, standard drugs including donepezil (5 mg kg^{-1}, p.o.) and methanolic extract of chickpeas (CPE) (1% carboxymethylcellulose (CMC), i.p.) were used. Rats were divided into three groups (n = 6) for each experiment and the test compounds and standard medications were administered for 14 days, with behavioral tests starting on the 14th day.

- For the control group or vehicle group, artificial cerebrospinal fluid (aCSF: 147 mM NaCl, 2.9 mM KCl, 1.6 mM $MgCl_2$, 1.7 mM $CaCl_2$ and 2.2 mM dextrose) was administered to rats
- To determine the most effective dose, a methanolic extract of CPS (100 and 200 mg kg^{-1}, p.o.) was tested in a streptozotocin-induced (STZ-IA) amnesia rat model, along with the administration of scopolamine (0.5 mg kg^{-1}, i.c.)
- In the experiment, standard drug donepezil DNP (5 mg kg^{-1}, p.o.) was used in a streptozotocin (STZ-IA) amnesia model along with the administration of methanolic extract of CPS (1% CMC, i.p.)

Surgery: On the day of surgery, rats were anesthetized with an i.p., injection of mixed chloral hydrate (300 mg kg^{-1}). The rats were then placed in a stereotaxic frame and bilateral stainless steel guidance cannulas (22-gauge) were inserted into the lateral ventricles according to the Paxinos Brain Atlas (AP 0.8, ML71.5, DV 3.5). The cannulas were secured to the skull using stainless screws and acrylic cement.

Streptozotocin Induced Amnesia (STZ-IA) model: To anesthetize rats, chloral hydrate (300 mg kg^{-1}, i.p.) was used. On days 1 and 3, a Hamilton Microsyringe was employed to slowly inject streptozotocin (STZ, 3 mg kg^{-1}) dissolved in a CSF into each lateral cerebral ventricle (i.c.v.) through a cannula placed at the following coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture and 3.6 mm ventral[21].

Behavioral testing: The water maze, described by Callan *et al.*[22], was a black circular pool that measured 140 cm in diameter and 70 cm in height. The water was filled to a depth of 25 cm and had a temperature range of 20 to 1°C. The maze was divided into four equal quadrants, with the release points for each quadrant labeled N, E, S and W. In the center of the southwest quadrant, a hidden circular platform (11 cm in diameter) was submerged 1.5 cm below the water's surface. Additional labyrinth visual signals, such as doors, windows, bookshelves, posters and computers, were fixed in various locations around the maze. To reduce the voltage over the center of the labyrinth, rat activity was first captured and then sent to a computer. Rats swimming along a path automatically recorded by computerized equipment (Noldus EthoVision, 3.1 version) had their path analyzed by computing various metrics, including swimming speed and platform.

Closed field activity: On days 13, 14 and 21 of i.c.v., STZ-IA, the rotational inertia activities were measured. The procedure involved placing each sample in a square closed arena (30 cm) equipped with infrared light-sensitive photocells and observing them for 5 min using a digital photoactometer Labgo, M.G Scientific traders, Haryana, India. The values were expressed as counts per 5 min. The other behavioral testing apparatuses were installed in darkened, light and sound-attenuated and ventilated testing areas. The passive avoidance behavior was evaluated first, followed by the transition latency in the plus maze and finally, the locomotor activity during the retest process on days 13, 14 and 21[23].

Biochemical studies

Tissue preparation: The rats were anesthetized using CO_2 inhalation, after which they were decapitated following the conclusion of the behavioral experiments. The hippocampi were promptly extracted and placed on ice, subsequently frozen using liquid nitrogen and stored at a temperature of 70°C.

To measure lipid peroxidation[24] proposed a method that involved adding 0.1 mL of processed tissue samples to 1.5 mL of acetic acid (20%, pH 3.5), 1.5 mL of thiobarbituric acid (0.8%) and 0.2 mL of sodium dodecyl sulfate (8.1%) in a sealed vial. The mixture was then heated at 100°C for 60 min, cooled with tap water and mixed with 5 mL of n-butanol:pyridine (15:1) and 1 mL of distilled water. The mixture was then centrifuged at 2000×g for 10 min and the resulting organic layer was measured for absorbance at 532 nm using a spectrophotometer (Marsap Services Private Limited, Mumbai, Maharashtra, India) he MDA concentration was expressed in nanomoles per gram of tissue[25].

Acetylcholinesterase (AChE) activity: Centrifugation at 100,000×g for 4 min at 4°C with 1% Triton X-100 (1%, w/v, in 0.01 M sodium phosphate buffer, pH 7.5) was performed on the brain homogenate 60 min using an ultracentrifuge manufactured by Beckman Coulter (Beckman Coulter, Inc., USA) the supernatant obtained was collected and then stored at 4°C for the subsequent measurement of AChE activity, utilizing Ellman's method[21]. The kinetics of AChE enzyme activity were determined spectrophotometrically at 412 nm with a 15 sec interval. The AChE activity was quantified as the number of micromoles (mol) of acetylthiocholine iodide hydrolyzed per minute per mg of protein, defining one unit of AChE activity. AChE-specific activity is expressed in µmol/min/mg of protein. The function of acetylcholinesterase (AChE) is vital for cholinergic processes and memory and alterations in AChE activity are linked to neurodegenerative conditions like dementia. In conditions such as Alzheimer's disease, cholinergic function is impaired due to the loss of cholinergic neurons and increased AChE activity. Monitoring AChE activity is vital to evaluate therapeutic interventions such as pumpkin seed oil (PSO), which can modulate cholinergic activity and improve memory and cognitive deficits in dementia models. Dysregulation of AChE is not limited to AD but can also occur in other neurodegenerative conditions.

Measurement of reduced glutathione: Proteins were segregated by spinning an equal volume of tissue homogenate combined with 10% trichloroacetic acid. Following this, 0.01 mL of the resulting supernatant was combined with 2 mL of a pH 8.4 phosphate buffer, 0.5 mL of 5-dithiobis (2-nitrobenzoic acid) and 0.4 mL of double-distilled water. After vortexing the mixture for 15 min, the absorbance at 412 nm was assessed to determine the quantity of reduced glutathione per gram of tissue[26-28].

Western blot analysis: Homogenization of hippocampi in a cold lysis buffer containing a protease and phosphatase inhibitor cocktail and then centrifuged the mixture at 14,000×g for 30 min at 4°C to extract debris. They used the Lowry method to determine the protein content of the samples and then loaded equivalent amounts of protein (40 mg) onto a 12% polyacrylamide gel electrophoresis gel. The gel was transferred to a PVDF membrane, which was then blocked in 5% BSA and probed overnight at 4°C with a 1/5000 dilution of primary antibodies (phospho GSK-3, GSK-3, phospho-Akt, Akt, caspase3, Bax, Bcl-2 and β-actin). They visualized the blots using the ECL pick kit and used ImageJ software to measure the band density[29].

Statistical analysis: The statistical methods employed for analyzing the data included repeated measures and one-way ANOVA. Tukey's multiple comparison test was utilized for comparison of the findings. The results are presented as Mean±SEM and a p-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

GC-MS analysis: Phytochemical profiling was performed using GC-MS analysis (4-hydroxy-3-methyl-2 butenyl acetate with retention time 6.291) in GC-MS research, dome inositol, stigmasterol, ergost-5-en-3-ol and ethyl alpha-d-glucopyranoside was also detected. The percentage peak area for stigmasterol (351125) was calculated.

Effect of CPE on behavioural parameters

Effect of CPE on streptozotocin induced amnesia model: Figure 1 Illustrated GC-MS data of chickpeas determines peak area and retention time of pharmacologically active compounds. Stigmasterol, ergost-5-en-3-ol and ethyl alpha-d-glucopyranoside was also detected.

Figure 2 illustrated the effects of CSA, STZ and CPE administration on spatial learning and memory in the water maze. The rats' ability to learn was depicted in Fig. 2a, which shows the escape latency to reach the secret platform. A repeated-measures analysis revealed a significant difference in escape latency between the groups ($p = 0.001$, $F = 15.162$; $df = 3, 31$). The STZ-receiving group had a higher escape latency compared to the vehicle-receiving group ($p = 0.001$). Additionally, a *post hoc* Tukey's test showed that the escape latency in the STZ-receiving group was significantly higher than in the vehicle-receiving group ($p = 0.001$). In Fig. 2b, the distance traveled to reach the secret platform is shown. The difference between groups was significant ($p = 0.001$, $F = 9.762$; $df = 3, 31$) according to the repeated measures analysis. The *post hoc* Tukey's test revealed that the travel distance of the STZ-receiving group was substantially greater than that of the vehicle-receiving group ($p = 0.001$). Furthermore, STZ-IA learning deterioration was reversed after treatment with 200 mg kg^{-1} CPE ($p = 0.018$). Finally, the effects of saline, STZ-IA and CPE administration on the mean swimming velocity during training days were shown in Fig. 2c. The swimming speed was not significantly dissimilar among the groups in a one-way ANOVA ($p = 0.6173$, $F = 0.6375$; $df = 3, 31$). The frequency of rat entry into the platform and its proximity were depicted in Fig. 2d. The results of the one-way ANOVA revealed a significant variation among the groups ($p = 0.0001$, $F = 6.031$; $df = 3, 31$). The frequency of entry into the platform region and its proximity was significantly reduced in the STZ-IA population, whereas CPE treatment at 200 mg kg^{-1} reversed memory impairment. Figure 2e shows the percentage of the total distance travelled in the goal

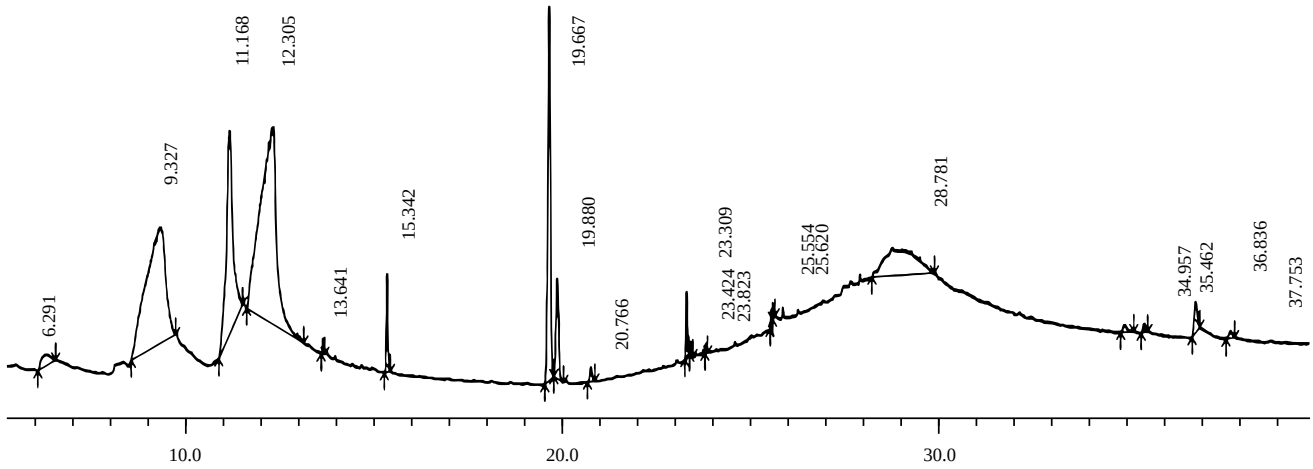

Fig. 1: Representative chromatogram of chickpeas used for identification of the bioactive molecule

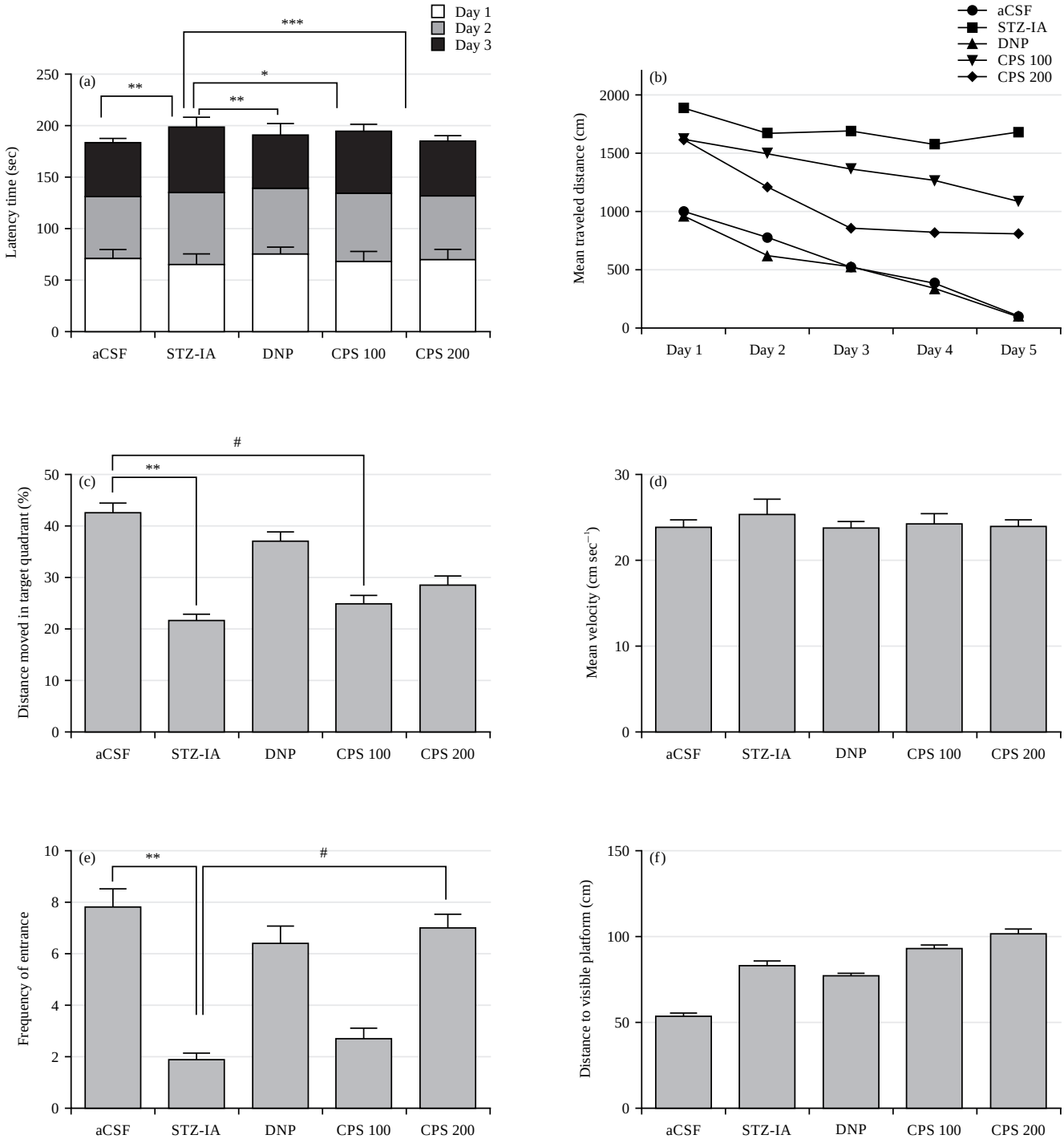

Fig. 2(a-e): Effect of CPS administration on spatial learning and memory on water maze, (a) Platform's escape latency, (b) Platform's arrival latency and depicts the distance traveled to reach the platform, (c) Average swimming speed over three days of preparation, (d) Extent to which the animals ventured into the platform and proximity, (e) Performance was impaired by STZ, but not reversed by 100 mg kg^{-1} of CPS. The 200 mg kg^{-1} of CPS did not differ from the control group and (f) Effect of saline, STZ-IA group and CPE on the success of the visible platform.

Data are expressed as mean +_ SEM (n=6) shows a significant increase in latency time, distance travelled time and distance to visible platform (P< 0.05, #p<0.01). In compare to control group and significant reduction (p< 0.05, #p<0.01) in compare to STZ group

region. One-way ANOVA indicated a significant difference among the groups ($p = 0.0014$, $F = 5.324$; $df = 3, 31$). The travel distance in the STZ-IA community was significantly reduced after *post hoc* analysis using Tukey's test. This test revealed that CPE at a dose of 100 mg kg^{-1} did not reverse the deficiency and there was no substantial difference between the control and CPE 200 mg kg^{-1}+STZ-IA groups, indicating that CPE at 200 mg kg^{-1} reversed the deficit. Figure 2f shows the effect of saline, STZ-IA and CPE on the success of the visible platform test, which measures the distance traveled to reach the visible platform. There were no significant differences in path length among the groups, indicating that STZ does not significantly affect sensorimotor synchronization or motivation in rats (one-way ANOVA, $p = 0.5874$, $F = 0.5345$; $df = 3, 31$). The CPE prevented STZ-induced memory loss, as there was no statistically significant difference in swimming speeds among any of the groups. The absence of an effect of STZ on the visible platform task suggests that the impairment was not caused by its influence on the rat's visual-motor or motivational systems[30]. The hippocampus plays a crucial role in spatial learning and memory and CPE may protect the hippocampus against STZ-IA's negative effects on hippocampal function[31].

Effect of CPE on locomotor activity: On days 13, 14 and 21, there was no significant disparity in spontaneous locomotor activity among the aCSF, STZ-IA and CPE (100 and 200 mg kg^{-1}) groups.

Effect of CPE on biochemical parameters

Effect of CPE on MDA level: The MDA levels in the rat brain were 532.1-61.0 nmol g^{-1} tissue in the STZ-IA group, significantly higher than 321.56-32.4 nmol g^{-1} tissue in the aCSF-treated group ($p<0.001$). The MDA levels in the STZ-IA group (262.2-39.2 and 197.2-28.1 nmol g^{-1} tissue, respectively; $p<0.001$) were substantially higher than in the CPE (200 mg kg^{-1}) groups. But the CPE (100 mg kg^{-1}) treated group showed no appreciable changes from the STZ-IA group (Fig. 3). Rats given CSF showed a rise in MDA, a lipid peroxidation marker, which suggests that their production of free radicals was elevated. Compared to STZ rats treated with aCSF, the rise in MDA levels in the brains of STZ-IA rats given 100 and 200 mg kg^{-1} CPE was less pronounced. The rats indicated a decrease in lipid peroxidation (Fig. 3).

Effect of CPE on glutathione levels: The levels of glutathione in the aCSF and STZ-IA-treated groups were 176.2-8.1 g g^{-1} tissue and 33.1-2.1 g g^{-1} tissue, respectively. Compared to the aCSF group, the STZ-IA group showed a significant ($p = 0.001$) decrease in glutathione levels. In comparison to the STZ-IA group (96.1-9.2 and 98.3-6.2 g g^{-1} tissue, respectively), the CPE (100 and 200 mg kg^{-1}) groups showed a significant ($p = 0.001$) increase in glutathione levels (Fig. 4). In STZ-IA rats, glutathione levels decreased significantly at the same time. Reduced glutathione is an anti-oxidant found in cells, with lower levels indicating increased free radical generation and depletion during oxidative stress. An extract antioxidant property may have increased glutathione levels in the CPE groups.

Effect of CPE on acetylcholinesterase enzyme levels: The administration of STZ alone resulted in a significant increase in activity in the STZ-IA group compared to the aCSF group (4.08 ± 0.284). Specifically, STZ administration led to an increase in AchE activity (8.82 ± 0.071; $p<0.001$). Additionally, compared to the STZ-treated group, CPE 100 mg kg^{-1} (3.51 ± 0.18), CPE 200 mg kg^{-1} (3.82 ± 0.23) and DNP (3.64 ± 0.541) all significantly reduced AchE levels. The AchE level reduction is promising for treating cognitive deficits in Alzheimer's disease, as it boosts cholinergic activity[32] (Fig. 5).

Rats given CPE following STZ did not show a significant difference in catalase levels compared to rats given STZ alone[33]. However, there was a notable difference in glutathione levels, suggesting that CPE's antioxidant properties reduce oxidative stress caused by STZ. The CPE ability to lower AChE levels and increase acetylcholine availability for better memory, as well as its anti-lipid peroxidative and antiepileptic qualities in a lithium pilocarpine model of septic shock, further support its memory-preserving properties against STZ-induced apoptosis. The CPE treatment also reduces hippocampus caspase-3 activation and MDA levels, which are associated with oxidative stress. The STZ component, nitrosamine, produces reactive oxygen species (ROS) that damage DNA. The CPE antioxidant characteristics may be responsible for lowering caspase-3 activation under oxidative stress[34], which helps prevent the onset of apoptosis. Studies both *in vivo* and *in vitro* have shown that CPE is effective in scavenging ROS, indicating that its antioxidant qualities play a role in its ability to reduce apoptosis[35].

Western blot analysis: Figure 6 displayed the effects of saline, STZ and CPE administration on caspase-3 cleavage in the rat hippocampus. The antibody against caspase-3 identified a 35 kDa band of procaspase-3 and one or two bands of cleaved caspase-3 at 19 and 17 kDa. The results of the one-way ANOVA indicated a significant difference between the groups ($p = 0.009$, $F = 10.344$; $df = 2, 11$). Although STZ-IA increased caspase-3 cleavage, CPE at a dose of 200 mg kg^{-1} reversed STZ-IA-induced caspase-3 cleavage, as shown by *post hoc* Tukey's test. Figure 6 depicts the effects of saline, STZ and CPE

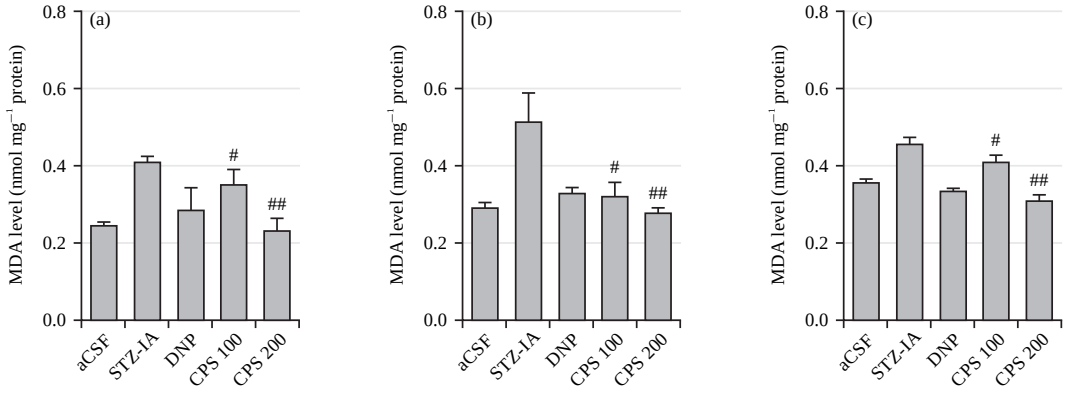

Fig. 3(a-c): CPS had a noticeable influence on the MDA levels in the brains of memory-impaired rats induced by STZ, (a) Cerebellum, (b) Hippocampus and (c) Cortex

Data represented as the Mean±SEM (n = 6), show a significant increase ($^{\#}p<0.05$, $^{\#\#}p<0.01$) in the MDA level in the control group compared to the STZ group, as well as a significant reduction ($^{\#}p<0.05$, $^{\#\#}p<0.01$) in the MDA level in the CPS group compared to the STZ group

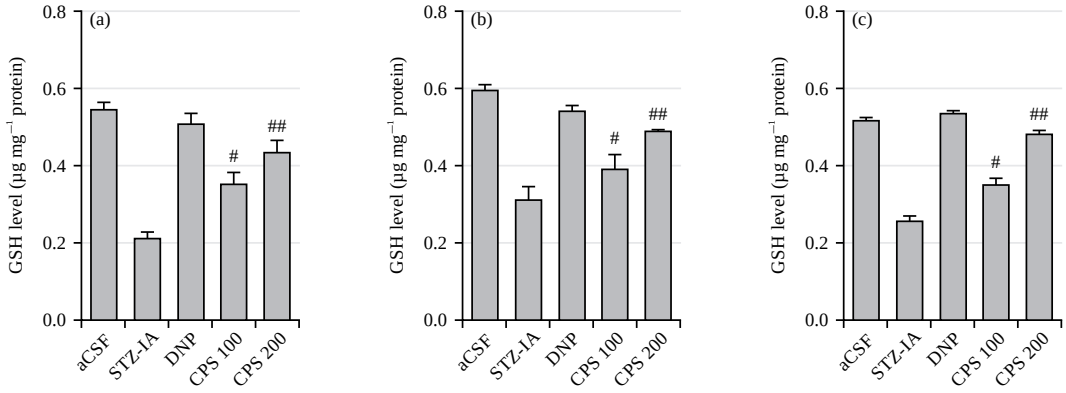

Fig. 4(a-c): Effect of CPS on GSH levels in the memory-impaired rat brain following STZ induction is depicted, (a) Cerebellum, (b) Hippocampus and (c) Cortex

Data are presented as the Mean±SEM (n = 6), results reveal a significant increase in GSH levels in the CPS-treated group compared to the control group, as well as a significant reduction compared to the STZ group ($^{\#}p<0.05$, $^{\#\#}p<0.01$)

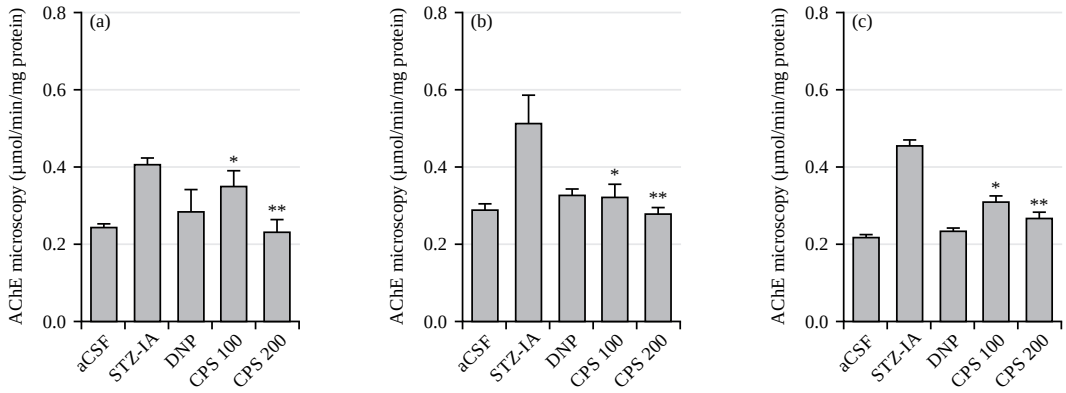

Fig. 5(a-c): Effect of CPS on AChE level in STZ induced memory impaired rat brain, (a) Cerebellum, (b) Hippocampus and (c) Cortex

Data are expressed as mean MDA level (nmol mg^{-1} protein)±SEM (n = 6). A significant increase (*p<0.05 and **p<0.01) compared to the control group and *significant reduction (*p<0.05 and **p<0.01) compare to the STZ group

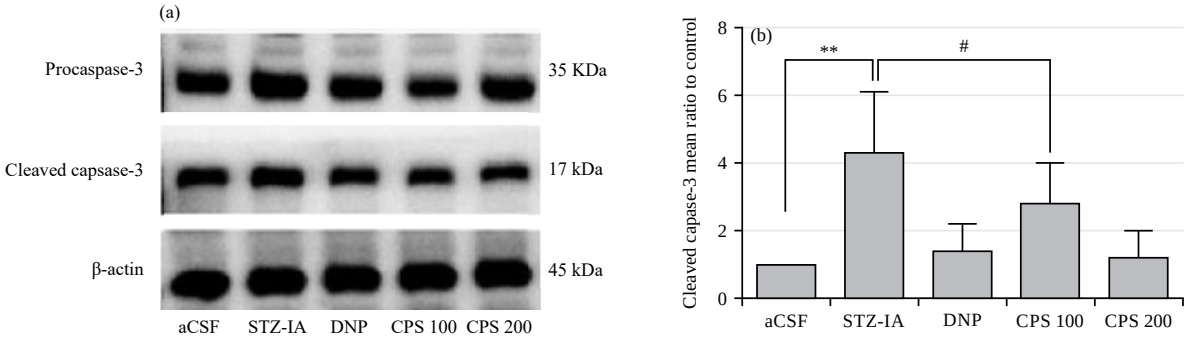

Fig. 6(a-b): Western blot analysis was conducted to find the effect of CPS in STZ-induced memory-impaired rat brains

Data are expressed as cleaved caspase 3 Mean±SEM (n = 6). A significant increase (**p<0.01) compared to the control group and a significant reduction ($^{\#}$p<0.05) compared to the STZ group

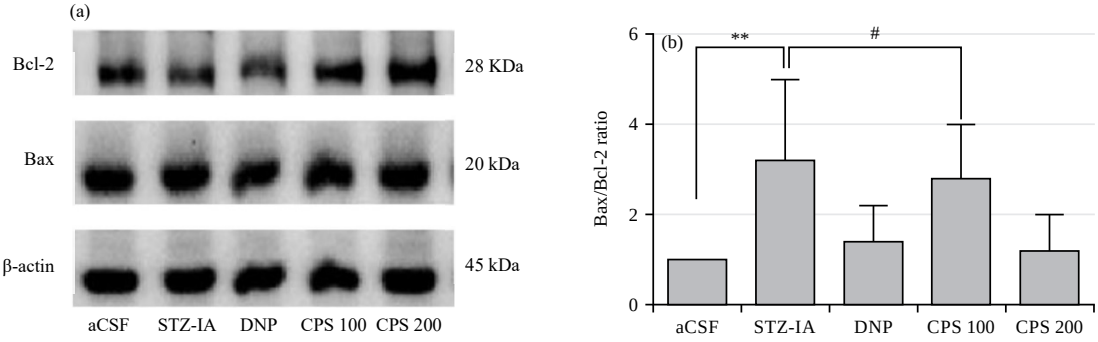

Fig. 7(a-b): Western blot analysis demonstrated the effect of saline, STZ and CPS on the hippocampal Bax/Bcl-2 ratio

Data are expressed as Bax/Bcl-2 ratio±SEM (n = 6). A significant increase (**p<0.01) compared to the control group and a significant reduction ($^{\#}$p<0.05) compared to the STZ group

administration on the Bax/Bcl2 ratio. The one-way ANOVA revealed a significant difference between the groups ($p = 0.0012$, $F = 8.781$; $df = 2, 11$). The STZ-IA increased the Bax/Bcl2 ratio, but CPE treatment at 200 mg kg^{-1} reversed this increase, as shown by *post hoc* Tukey's test. Figure 7 illustrated the effect of saline, STZ and CPE administration on phosphorylated Akt in the hippocampus, as determined by western blotting. The results of the one-way ANOVA revealed a significant difference between the groups ($p = 0.005$, $F = 5.792$; $df = 2, 11$). The STZ-IA suppressed Akt activation, while CPE therapy at 200 mg kg^{-1} reversed STZ-IA-mediated suppression of Akt activation. Finally, Fig. 7 shows the effect of saline, STZ and CPE administration on the GSK3 activity index. The one-way ANOVA revealed a significant difference between the groups ($p = 0.0003$, $F = 28.139$; $df = 2, 11$). The STZ-IA decreased the GSK3 activity index, but CPE treatment at 200 mg kg^{-1} reversed this decrease. The STZ treatment increased the GSK3/p-GSK3 ratio, according to a *post hoc* Tukey's test. The STZ-induced GSK3/p-GSK3 elevation was reversed by CPE treatment at 200 mg kg^{-1} (Fig. 8). Several protein families, including Bax and Bcl-2, regulate apoptotic cell death. The Bcl-2 is anti-apoptotic, whereas Bax is pro-apoptotic and the relative expression of these two proteins influences cell viability[36]. The STZ decreased Bcl-2 and increased Bax levels, but CPE restored them and activated STZ-IA caspase-3. The exact mechanism is unknown, but CPE antioxidant properties are speculated to play a role. Alternatively, activation of the PI3/Akt pathway may be responsible for changes in Bcl-2 family expression, suggesting that CPE may indirectly increase Bcl-2 by restoring Akt activity[37]. The STZ-IA limits the effects of systemic insulin on the hippocampus by reducing blood-brain barrier permeability. The Akt, also known as PKB, is a serine/threonine kinase that mediates PI3K's downstream effects on insulin signaling. Intracerebroventricular insulin administration induces Akt phosphorylation[38], but in current samples, STZ treatment decreased phospho-Akt levels due to its negative effect on insulin signaling. The Akt's direct target is GSK-3, which is strongly expressed in the brain. The Akt phosphorylates GSk-3 at Ser-9, inhibiting its kinase activity[39,40].

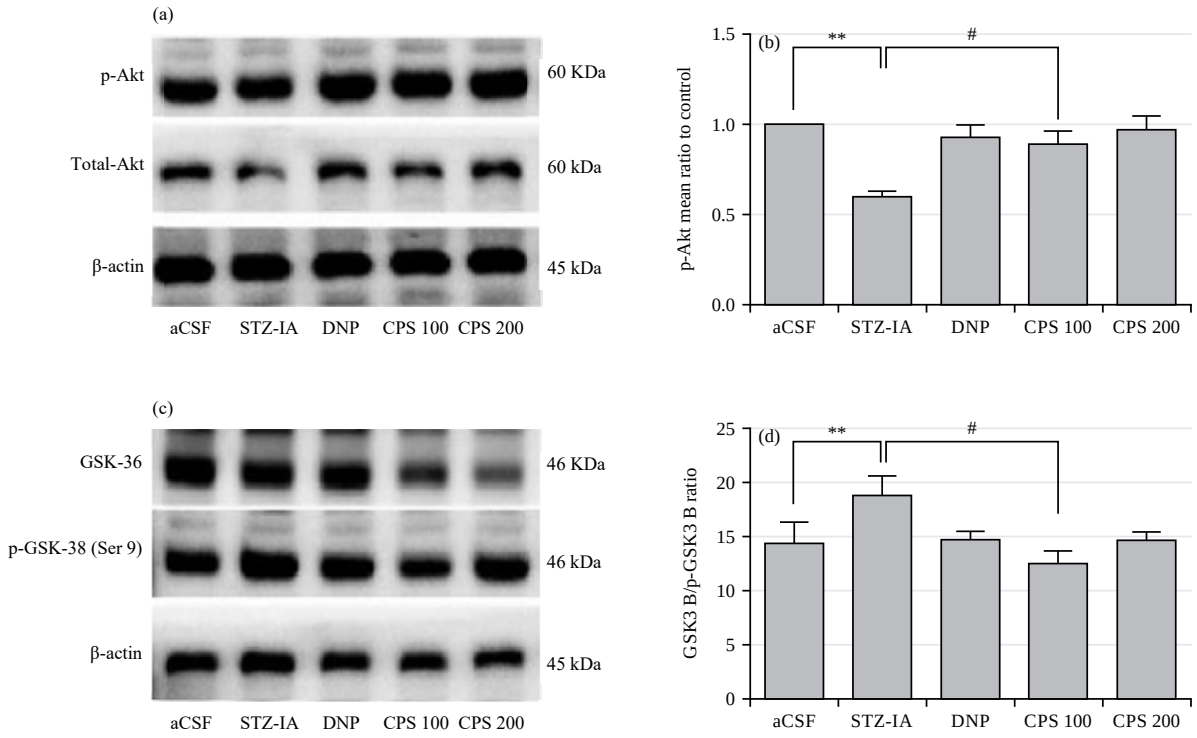

Fig. 8(a-d): Western blot analysis demonstrated the effect of saline, STZ and CPS on the hippocampal GSK3B/p-GSK3B ratio

Data are expressed as GSK3B/p-GSK3B±SEM (n = 6). A significant increase (**p<0.01) compared to the control group and a significant reduction ($^{\#}$p<0.05) compared to the STZ group

The western immunoblots were probed with antibodies against phosphorylated Akt, total Akt (Fig. 8a-b), GSK-3β and β-actin (Fig. 8c-d). The result revealed a statistically significant difference between the control group and the group that received STZ (p<0.01). Furthermore, the analysis showed a noticeable difference between animals that received STZ and CPS-treated groups.

The key intermediate step in determining whether a novel treatment will work in human patients is to determine whether or not memory is improved in a relevant animal model. The studies discussed in this review demonstrate the progress that has been made in understanding the anatomical and physiological mechanisms underlying memory and their relevance to human dementias. Continuing improvements in the validity of the available animal models to test novel drugs should improve our ability to discover and develop new treatments for AD and other dementias.

CONCLUSION

This is the first study to demonstrate that administration of 200 mg kg^{-1} of CPE can modulate apoptosis-related proteins such as caspase-3, Bax and Bcl-2 in the hippocampus and restore centrally given STZ-IA. Additionally, this research shows that CPE can reverse the Akt/GSK3β disruption caused by STZ-IA, which may help explain its neuroprotective effects. The findings of this study may contribute to the development of effective treatment strategies for senile dementia and Alzheimer's disease due to the similarities between central STZ-IA and AD.

SIGNIFICANCE STATEMENT

The study exposed the improvement of learning and memory by chickpeas (*Cicer arietinum* L.) sprout against streptozotocin and induced amnesia in rats. It showed a neuroprotective effect by decreasing acetylcholinesterase enzyme and by increasing GSH in rat models. As chickpeas sprout is a good source of nutrition. Thus, a novel approach that chickpeas sprout is effective and safe to treat a patient suffers from Alzheimer' s disease may be arrived at.

ACKNOWLEDGMENT

The authors are thankful to Hygia Institute of Pharmaceutical Education and Research, Lucknow, India.

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