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Research Article

EVALUATION OF PROTECTIVE EFFECT OF CURCUMIN AGAINST CYCLOPHOSPHAMIDE-INDUCED COGNITIVE IMPAIRMENT IN SWISS ALBINO MICE

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ABSTRACT

Objectives: The objective of the present study was to evaluate the effect of curcumin against cognitive dysfunctions associated with the chemotherapeutic agent cyclophosphamide (CYP) in Swiss albino mice.

Methods: The symptoms of chemobrain in Swiss mice were observed by the administration of CYP 50 mg/kg body wt. intraperitoneally once daily for 3 weeks. Oral treatment of test drug curcumin at doses of 200 and 400 mg/kg body wt. was administered daily for 3 weeks. Donepezil was used as the standard drug at 3 mg/kg body wt. orally. Assessment of memory dysfunction was done by Morris water maze, passive avoidance test, and elevated plus maze test. Anti-oxidant parameters were also checked from the isolated brain samples.

Results: It was found that the CYP significantly (***p<0.001) reduced the cognitive ability in Swiss albino mice based on behavioral and anti-oxidant parameters, whereas the animals treated with curcumin were significantly (**p<0.01) protected from behavioral cognitive abnormalities. The anti-oxidant parameters of the brain also support the behavioral findings.

Conclusion: The data obtained from this study proved that curcumin has the ability to protect the CYP-induced cognitive dysfunction and could be a promising treatment strategies to protect the chemobrain.

Keywords: Cyclophosphamide, Curcumin, Cognitive impairment, Chemobrain.

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INTRODUCTION

Cognitive impairment refers to an alteration in mental functions such as memory, concentration, language, and problem-solving abilities [1]. This condition can range from mild cognitive impairment, where individuals experience noticeable but manageable deficits, to severe forms such as dementia, which significantly interfere with daily activities [2]. Cognitive impairment results from degeneration of cholinergic neurons. Memory impairment is generally the prominent sign and symptom in Alzheimer's disease, Parkinson's disease, traumatic brain injuries, and psychiatric disorders [3]. Early diagnosis and intervention are crucial for managing symptoms and slowing progression [4]. Therapeutic strategies may include pharmacological treatments, cognitive training, and lifestyle modifications aimed at promoting brain health [5]. Prevalence of cognitive impairment is expected to rise globally, underscoring the need for effective public health strategies and support systems [6].

Cyclophosphamide (CYP), a cytotoxic alkylating drug, is frequently used as an immunosuppressive medication for organ transplantation as well as a chemotherapeutic medicine for the treatment of different cancers. Nevertheless, CYPs have so many adverse reactions on the human body; one of them is cognitive deficit in patients with cancer. Its metabolites are also responsible for creating alkylation on the DNA strands of proliferating cells, which leads to their apoptosis. The blood–brain barrier (BBB) can be crossed by it. CYP is an immunosuppressive and anti-cancer drug. As a result of bioactivation, the parent drug (CYP) or "pro-drug" produces phosphoramide mustard and acrolein, which alkylate DNA and W proteins [7].

Based on a scientific literature survey, it was found that there is a lack of information regarding the protective role of curcumin in CYP-induced cognitive impairment. Despite the seriousness of the cognitive impairment that occurs due to chemotherapeutic agents, there are no therapeutic drugs currently available for its management. The aim of this study was to assess the effect of curcumin against CYP-induced cognitive impairment in Swiss albino mice.

METHODS

Drugs and chemicals

Analytical grade reagents and chemicals were utilized in this study. Curcumin, as a test drug procured from S D Fine-Chem. Ltd., Worli Road, Mumbai, CYP, and donepezil were obtained from Cadila Pharmaceuticals.

Experimental animals

Swiss albino mice (20–25 g) were used in this study after approval from the Institutional Animal Ethics Committee (IAEC), Babu Banarasi Das Northern India Institute of Technology (BBDNIIT), Lucknow. (IAEC approval number BBD/IAEC/81/02/2020.) Animals were obtained from the animal house of BBDNIIT Lucknow. Housing conditions for animals were maintained as per the guidance of the Committee for Control and Supervision of Experiments on Animals.

Experimental design

After acclimatization of 1 week, all the animals were randomly allocated to the five different treatment groups, each group containing five animals, as shown in Table 1.

Behavioral parameters

Modified passive avoidance (MPA)

Long-term memory was assessed using the step-down test, which consisted of a 5 min training session followed by a 5 min test session,

Table 1: Grouping and treatments of animals for pharmacological study

Groups	Treatments	No. of animals
Normal control	Vehicle (1% CMC suspension) 10 mL/kg p.o	5
Disease control	Cyclophosphamide (50 mg/kg/week i.p.) for 3 weeks	5
Standard	Donepezil (3 mg/kg OD, for 3 weeks p.o)+cyclophosphamide (50 mg/kg/week i.p.) for 3 weeks)	5
Test 1	Cyclophosphamide (50 mg/kg/week i.p. for 3 weeks)+curcumin suspension (100 mg/kg body wt. OD, for 3 weeks)	5
Test 2	Cyclophosphamide (50 mg/kg/week i.p. for 3 weeks)+curcumin suspension (200 mg/kg body wt. OD, for 3 weeks.	5

CMC: Carboxymethylcellulose

acquisition work 2 h later, and retention work 24 h later. The chamber's measurements were roughly 40 (h), 33 (w), and 35 (d) cm. The chamber's floor was made out of a grid of parallel, 0.1 cm thick copper bars. On the 21st day, 1 h after dosing, animals were carefully placed onto the wooden platform of 1.7 cm high, 12 × 15 cm, throughout the training session facing toward the bottom corner. The mice immediately felt an electric shock after placing their paws on the electrified grid (0.2mA, 5 s). They exhibited an innate propensity to leap up the platform in order to avoid the shock. The number of times the mice stepped down throughout the training session (error counts) and the length of time it took the mouse to step down from the platform onto the four grid (known as "Step Down Latency" [SDL]) were both noted. The same steps were taken during the test session. To lessen the chance of odor interference, the apparatus was thoroughly cleaned after the test session [8].

Elevated plus maze (EPM) test

EPM consisted of two closed arms (25 cm × 8 cm × 15 cm) and two open arms (25 cm × 8 cm) connected to a central platform (5 cm × 5 cm). The maze was elevated to a height of 24 cm above the floor. All the treatments were given orally. The time spent and the number of entries in both the open and closed arms were recorded for 5 min. On the first day, each mouse was placed at the end of the open arm, facing away from the central platform. The time spent and the number of entries in both the open and closed arms were recorded for 5 min. The time spent is measured in seconds. An entry was defined as having all four paws of animals within the arm [8].

Morris water maze (MWM)

The apparatus was a 1.5 m in diameter and 35 cm deep circular pool filled to a level of 25 cm with water (at 30°C). With the aid of two threads that were fixed at a straight angle to each other on the pool's rim, the tank was divided into four equal quadrants. A circular platform (10 cm × 11.5 cm, 20 cm tall, with a top surface 7 cm × 7 cm and painted 5 cm in white) was placed in one quadrant (Q4 in the present study) of the pool, 1 cm above the water level during the acquisition phase. Each animal underwent 4 days of four trials per day, separated by 5 min each (starting from the 19th day of drug administration to 21 day).

Maximum swim time was set to 120 s. If the mouse could not locate the platform after 120 s, it was gently directed there and given 20 s to stay. The amount of time it takes an animal to swim from the starter quadrant to the target quadrant (TQ), where the hidden platform is located, is known as the "escape latency" (EL). EL is used as an indicator of learning or acquisition.

The average amount of time spent in the TQ in search of the hidden platform was recorded as a retrieval or memory index. EL, the number of crossings and residence time was evaluated [8].

Biochemical investigation

Tissue homogenization

All the animals were sacrificed through cervical dislocation. To prevent tissue damage, the entire brain was carefully removed, placed in an ice bath, and cleansed with an isotonic saline solution. The tissue was washed with normal saline (0.9% 3 w/v) and then quickly homogenized in ice-cold phosphate-buffered saline (pH 7.4). The supernatant from the centrifugation of the homogenate for 30 min at 4,000 rpm was kept at 40°C and utilized to test the biochemical parameters.

Estimation of malondialdehyde (MDA) or lipid peroxidation (LPO)

MDA in brain tissues was quantified using the Colado methodology (1997). After adding 300 µL of 30% trichloroacetic acid (TCA), 150 µL of 5N HCl, and 300 µL of 2% w/v 2-thiobarbituric acid to 500 µL of tissue homogenate in phosphate buffer (pH 7.4), the mixture was heated for 15 min at 90°C. The mixture was centrifuged for 10 min at 12,000 g. A pink-colored supernatant was obtained, and its wavelength, 532 nm, was determined spectrophotometrically. Using 1,1,3,3-tetraethoxypropane as a standard, the MDA concentration was calculated and reported as nmoL/mg protein [9].

Estimation of glutathione (GSH)

After mixing the 500 µL brain homogenate with an equal volume of 10% TCA, the mixture was centrifuged (using a Remi cold centrifuge) at ×2000 g for 10 min at 4°C. The supernatant was utilized to estimate GSH. Add 0.4 mL of double-distilled water, 0.5 mL of DTNB (0.2% in 0.1 M sodium phosphate buffer, pH 8.4), and 2 mL of phosphate buffer (pH 8.4) to 0.1 mL of processed tissue sample, and the mixture was shaken vigorously on a vortex. Within 15 min, the absorbance was read at 412 nm. GSH was used as a standard to assess GSH concentration, which was expressed as µg/mg protein [10].

Estimation of catalase (CAT) level

For the assessment of CAT level in tissues, 500 µL of tissue homogenate was subjected to 1950 µL of 50 mM phosphate buffer at pH neutral and 1000 µL of H_2O_2, 30 mM. At 240 nm, the absorbance was measured 3 times at intervals of 15 s. The absorbance at 0–30 s was subtracted to determine the real absorbance. Utilizing the millimolar extinction coefficient of H_2O_2, i.e., 0.071 mmoL cm^{-1}, the result was calculated. The activity was measured in terms of µmoL of H_2O_2 broken down per min per mg of protein [11].

Estimation of superoxide dismutase (SOD) level

SOD activity was estimated by the method of Ghadrdoost *et al*. Reaction mixture of this method contained: 50 mM sodium carbonate (1.56 g/mL), 96 µM Nitro blue tetrazolium, Triton-X-100 (v/v) in reagent, 20 mM of hydroxylamine hydrochloride (1.38 mg/10 mL) pH-6, 0.3 mL of the supernatant after centrifugation (×1500 g for 10 min followed by ×10,000 g for 15 min) of homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 mL of NADH (780 µmoL) and stopped after 1 min by adding 1 mL of glacial acetic acid. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed in units/mg protein [12].

Statistical analysis

For the analysis of study data, GraphPad Prism version 5 was used. All the observed data were expressed as Mean±SEM. The data were analyzed by an analysis of variance (ANOVA), one-way ANOVA followed by Tukey post-test.

RESULTS

Behavioral assessment

EPM

Here, the effect of treatment on the cerebral palsy group, test group, and standard group (3 mg/kg) was evaluated at the end of the treatment

(Table 2). The CYP treated group showed a considerable ($p<0.0001$) increase in TL values on the day of training (acquisition day) as well as on the day of retention, indicating impairment in learning and cognitive ability (Figs. 1 and 2). However, the animals treated with curcumin significantly ($p<0.05$, $p<0.0001$) reduced the transfer latency values on day 22 when compared to the CYP treated group. The results observed with the test drug are close to the standard drug.

MPA

Administration of CYP (CYP 50 mg/kg, i.p) reduces SDL on acquisition day 21 and test latency, indicating memory impairment. A significant decrease in SDL and an increase in step down error (SDE) indicate the cognitive impairment caused by CYP, as depicted in Tables 3 and 4 Figs. 3-6. An increase in SDL and a decrease in SDE in Test 1 and Test 2 at both low and high doses showed that the test compound is able to protect the CYP-induced memory impairment. Animals of the standard group treated with donepezil (3 mg/kg, i.p) showed a significant protection in cognitive impairment induced by CYP.

MWM test

EL in MWM

Latency of escape for the animals treated with CYP was significantly higher than the normal control group on the 21st day of treatment, and this difference also began on the 2nd day. The standard drug, as well as the test drug, reversed the cognitive dysfunction effect of CYP on the final test day and significantly 5 ($p<0.001$, $p<0.01$) enhanced memory performance. Curcumin-treated animals have taken less time to reach the hidden platform as compared to the disease control (CYP treated) group, showing an increase in learning and memory ability in mice (Table 5).

Residence time (time spent in TQ) in MWM test

Animals treated with CYP showed a decreased time to stay in TQ as compared with the normal control animal, which signifies the cognitive impairment.

Table 2: Effect of test 1 and test 2 treatment on transfer latency in CYP-induced memory impairment in mice

Groups	Transfer latency (seconds)	
	Acquisition day 21	Retention day 22
Normal control	20.8±0.78	14.40±0.76
Disease control	31.04±0.89***	39.20±0.72***
Standard	23.80±0.59***	17.80±1.17***
Test 1	28.20±0.72*	26.20±1.34***
Test 2	25.20±0.56***	22.80±0.57***

CYP: Cyclophosphamide. Result expressed as mean±SEM (n=5). *p<0.05, **p<0.01, ***p<0.001 when compared with the disease control group

Table 3: Effect of test 1 and test 2 on step down latency in modified passive avoidance in CYP-induced memory impairment in mice

Groups	Step down latency (seconds)	
	Step down latency (Day 21)	Step down latency (Day 22)
Normal control	41.8±1.32	60.80±1.03
Disease control	23.20±0.81***	29.60±0.68***
Standard	34.20±0.80***	42.8±1.14***
Test 1	26.80±0.72	32.2±0.78
Test 2	29.20±0.59***	35.8±1.39*

CYP: Cyclophosphamide. Result expressed as mean±SEM (n=5). *p<0.05, ***p<0.001 when compared with the disease control group

Animals treated with the test drug at 200 mg/kg for 21 days showed considerable increase ($p<0.01$**) in time spent in the TQ when compared to diseased control group (Table 6 and Figs. 7 and 8) whereas the standard drug donepezil showed maximum increase in residence time ($p<0.001$***).

Biochemical investigation

Effect of test 1 and test 2 on CAT activity in CYP-induced cognitive impairment

CAT level in the brain homogenate of animals treated with CYP was found to be decreased in comparison to the normal control group animals treated with vehicle, i.e., carboxymethylcellulose (CMC). Animals treated with test drug for 21 days (curcumin at 200 mg/kg, p.o) showed a considerable increase (*$p<0.05$) in brain CAT level when compared to the diseased control group. However, the animals treated with donepezil (3 mg/kg) for 21 days showed a maximum increase in CAT level as depicted in Table 7 and Fig. 9.

Effect of test 1 and test 2 on MDA activity in CYP-induced cognitive impairment

MDA level in the brain homogenate of animals treated with CYP was found to be elevated in comparison to the normal control group animals treated with vehicles, i.e., CMC.

Table 4: Effect of test 1 and test 2 on step down error in CYP-induced memory impairment in mice

Groups	Step down error (Number)	
	Step down error (Day 21)	Step down error (Day 22)
Normal control	5.6±0.37	2.6±0.19
Disease control	8.8±0.25***	6.2±0.32***
Standard	6.6±0.30**	4.4±0.51*
Test 1	7.8±0.43	7.2±0.3
Test 2	6.4±0.38***	5.6±0.38

CYP: Cyclophosphamide. Result expressed as mean±SEM (n=5). *p<0.05, **p<0.01, ***p<0.001 when compared with the disease control group

Table 5: Effect of test 1 and test 2 on step down error in CYP-induced memory impairment in Morris water maze test

Groups	Acquisition latency (sec)		Retention latency (sec)
	Day 19	Day 20	Day 21
Normal control	46.80±0.84	36.20±0.74	31.40±0.88
Diseased control	73.40±0.93	71.40±1.01	68.20±0.95
Standard	49.80±0.52	45.20±0.78	41.20±0.67**
Test 1	71.00±0.95	69.20±0.77	63.00±1.12
Test 2	69.80±1.10	66.40±0.48	62.60±0.81**

CYP: Cyclophosphamide. Result expressed as mean±SEM (n=5). *p<0.05, **p<0.01, ***p<0.001 when compared with the disease control group

Table 6: Effect of test 1 and test 2 on time spent in target quadrant in CYP-induced memory impairment in Morris water maze test

Groups	TSTQ (sec.)
Normal control	21.40±0.77
Diseased control	9.20±0.38
Standard	18.20±0.48***
Test 1	10.80±0.38
Test 2	12.20±0.52**

CYP: Cyclophosphamide, TSTQ: time spent in target quadrant. Result expressed as mean±SEM (n=5). **p<0.01, ***p<0.001 when compared with the disease control group

Table 7: Effect of test 1 and test 2 on catalase, MDA, GSH, and SOD in CYP-induced memory impairment in mice

Groups	Catalase (U/min/mg protein	MDA (nmoL/mg protein)	GSH (nmoL/mg protein)	SOD (U/mg protein)
Normal control	3.74±0.10	2.54±0.65	8.36±0.59	1.62±0.04
Diseased control	0.99±0.17	6.17±0.54	3.26±0.65	0.89±0.02
Standard	2.01±0.25**	3.03±0.35 **	7.32±0.68**	1.11±0.03**
Test-1	1.18±0.19	5.92±0.54	5.66±0.86	0.75±0.04*
Test-2	1.79±0.15*	4.11±0.23 *	7.04±0.94*	1.09±0.03**

MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, CYP: Cyclophosphamide. Result expressed as mean±SEM (n=5). *p<0.05, **p<0.01, ***p<0.001 when compared with the disease control group

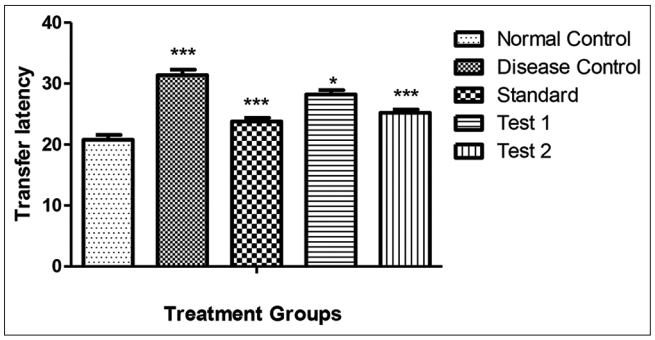

Fig. 1: Effects of test 1 and test 2 on transfer latency in cyclophosphamide-induced memory impairment in mice on Day 21

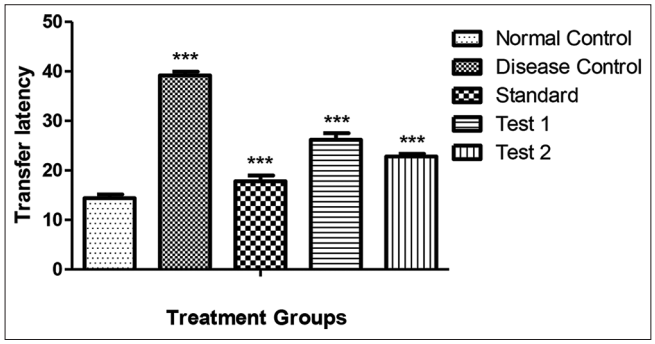

Fig. 2: Effects of test 1 and test 2 on transfer latency in cyclophosphamide-induced memory impairment in mice on Day 22

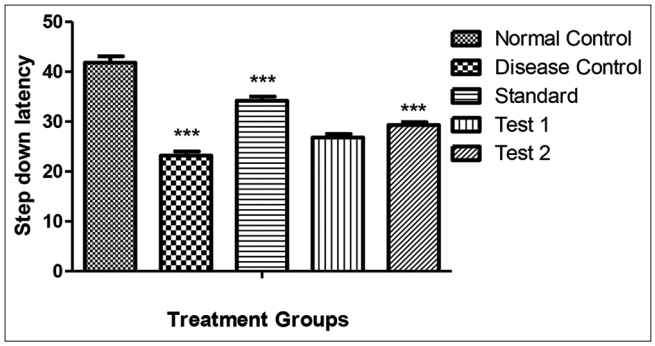

Fig. 3: Effects of test 1 and test 2 on step down latency in cyclophosphamide-induced memory impairment in mice on Day 21

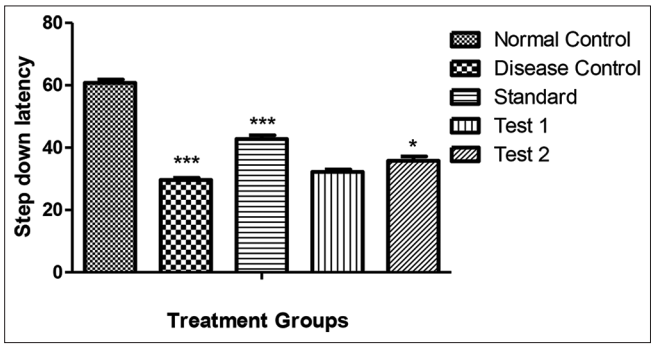

Fig. 4: Effects of test 1 and test 2 on step-down latency in cyclophosphamide-induced memory impairment in mice on Day 22

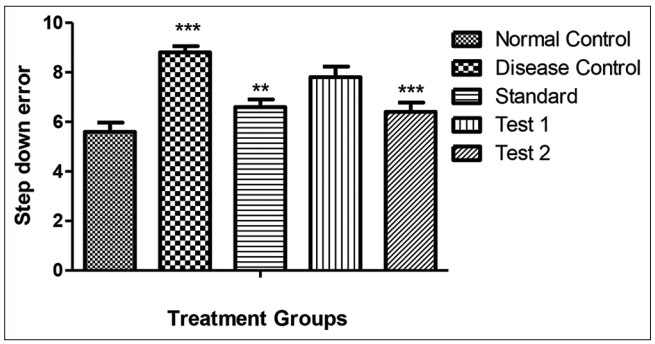

Fig. 5: Effects of test 1 and test 2 on step-down error in cyclophosphamide-induced memory impairment in mice on Day 21

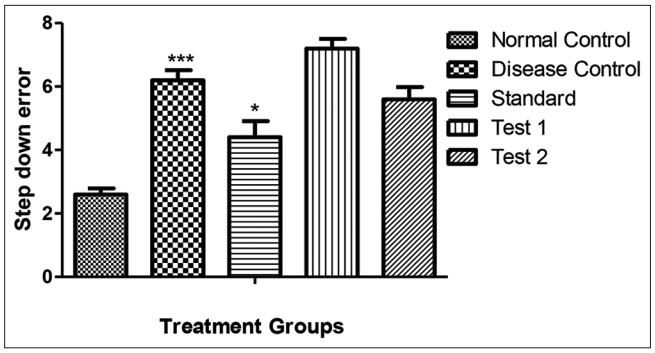

Fig. 6: Effects of test 1 and test 2 on step-down error in cyclophosphamide-induced memory impairment in mice on Day 22

Animals treated with test drug for 21 days (curcumin at 200 mg/kg, p.o) showed considerable restoration (*p<0.05) in brain MDA level when compared to the diseased control group. However, the animals treated with donepezil (3 mg/kg) for 21 days showed maximum effect in restoration of MDA level, as depicted in Table 7 and Fig. 10.

Effect of test 1 and test 2 on GSH activity in CYP-induced cognitive impairment

GSH level in the brain homogenate of animals treated with CYP was found to be reduced in comparison to the normal control group animals treated with vehicles, i.e., CMC.

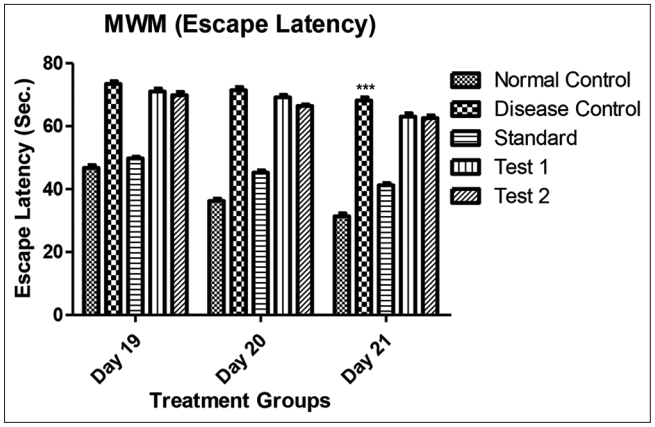

Fig. 7: Effects of Test 1 and test 2 on escape latency in cyclophosphamide-induced memory impairment in mice

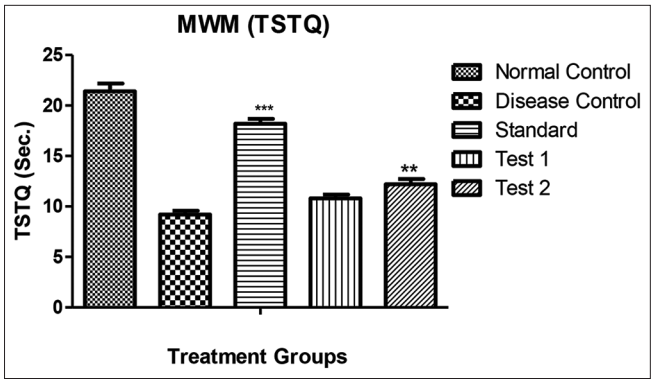

Fig. 8: Effect of test 1 and test 2 on time spent in the target quadrant in cyclophosphamide-induced memory impairment in the Morris water maze test

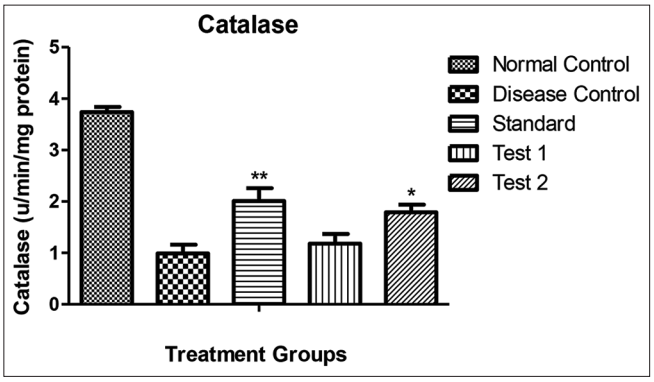

Fig. 9: Effect of test 1 and test 2 on catalase activity in cyclophosphamide-induced memory impairment in mice

Animals treated with test drug for 21 days (curcumin at 200 mg/kg, p.o) showed a considerable increase (*p<0.05) in brain CAT level when compared to the diseased control group. However, the animals treated with donepezil (3 mg/kg) for 21 days showed a maximum increase in GSH level, as depicted in as depicted in Table 7 and Fig. 11.

Effect of test 1 and test 2 on SOD activity in CYP-induced cognitive impairment

SOD level in the brain homogenate of animals treated with CYP was found decreased in the comparison of normal control group animals treated with vehicles, i.e., CMC.

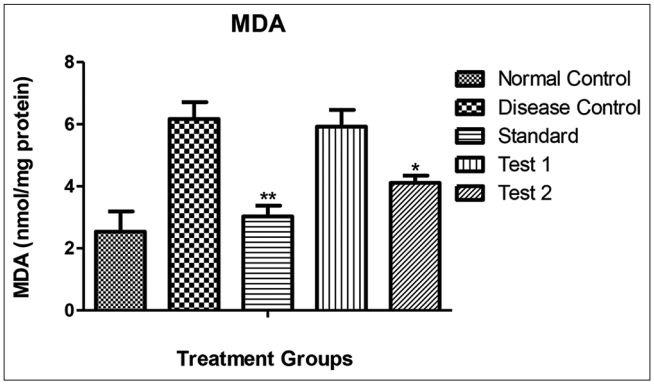

Fig. 10: Effect of test 1 and test 2 on malondialdehyde activity in cyclophosphamide induced memory impairment in mice

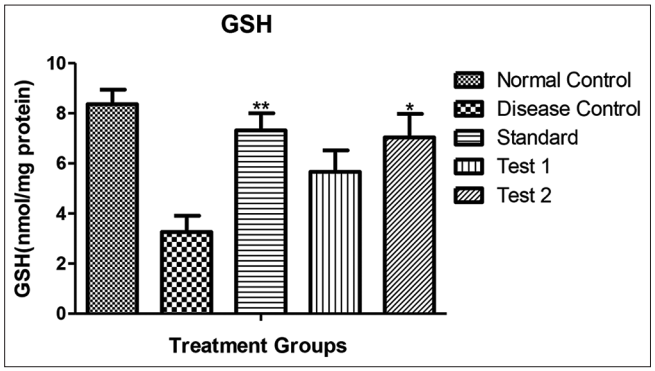

Fig. 11: Effect of test 1 and test 2 on glutathione activity in cyclophosphamide-induced memory impairment in mice

Animals treated with test drug for 21 days (curcumin at 200 mg/kg, p.o) showed considerable increase (**p<0.01) in brain SOD level when compared to diseased control group and similar to the animals treated with donepezil (3 mg/kg) for 21 days in Table 7 and Fig. 12.

DISCUSSION

Impaired memory is a loss of intellectual capacity that severely affects a person's ability to perform routine jobs, engage in regular social interactions, or maintain close relationships, provided there is no major impairment of awareness or involvement of the motor system. For both cancer patients and those in remission, cognitive impairment has an adverse impact on routine life functioning, quality of expected life, and job potential. Various pharmacological models were utilized with the Swiss albino mice model in the current investigation to evaluate the protective potential of curcumin on CYP-induced cognitive impairment. EPM, MWM, and MPA are three separate behavioral assessment models used in this study. One of the most commonly used chemotherapy drugs is CYP, primarily indicated to treat malignant lymphomas and multiple myelomas, ovarian adenocarcinomas, breast cancer, and retinoblastoma. Along with its anti-cancer effects, it is also having immunosuppressive properties that prevent transplant rejections in autoimmune diseases. CYP penetrates the BBB, and the dyscognition it causes may be brought about by reactive oxygen species (ROS) production and oxidative damage that directly harm neurons, or indirect tissue toxicity. The genome cannot be replicated because it alkylates DNA. In addition, CYP inhibits cell growth and promotes cell death. Acrolein/phosphoramide mustard, a CYP metabolite that causes oxidative stress and damages the BBB, allows neurotoxic chemicals to enter the brain [13].

In the MPA test, the SDL test, and retention transfer latency. The retention transfer latency was significantly reduced in the animals

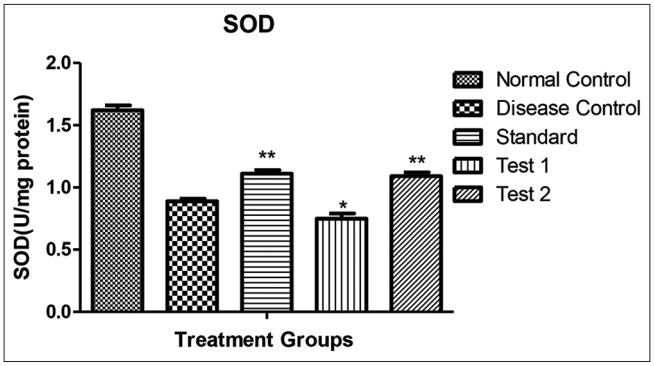

Fig. 12: Effect of test 1 and test 2 on superoxide dismutase activity in cyclophosphamide-induced memory impairment in mice

treated with CYP, as shown in Tables 3 and 4 [13]. Animals were provided a single dose of CYP once a week, and there was a significant increase in the levels of nitrites, hydrogen peroxide production, and MDA content. In addition, the levels of anti-oxidant enzymes CAT, SOD, and GSH were decreased in the homogenate of brain, as shown in Table 8. When spatial memory was tested using MWM, it was discovered that the CYP-treated group took far longer to get to the escape platform in comparison to the control group, as depicted in Tables 5 and 6 [14,15]. Donepezil falls under the category of nootropic substances in a broader sense. Donepezil has been demonstrated to change the physical characteristics of the plasma membrane and protect the cell death in hypoxia. It promotes the deformability of red blood cells and restores normal platelet aggregation. Donepezil is a substance with rheological, neuroprotective, and antithrombotic effects. The ability of donepezil to treat conditions including dementia, vertigo, myoclonus, and stroke may be due to this molecule's interaction with the membrane phospholipids, which restores membrane fluidity. Drugs that modulate cerebral functions include donepezil and donepezil-like substances. These medications are also used to treat encephalopathies caused by a variety of causes, such as cranial trauma, inflammation, and complications from stroke or ischemia following bypass surgery. Some derivatives are also used to treat neurological conditions such as seizures and neuromuscular convulsions [16]. In India turmeric is utilized traditionally as an analgesic and anti-inflammatory agent which contains curcumin.Curcumin has been shown to improve cognition and effectively treat brain disorders. Curcumin is a powerful anti-oxidant that is excellent at neutralizing free radicals, preventing LPO, and xanthine oxidase activity. Curcumin can reduce behavioral dysfunction and memory loss during the progression of neurodegenerative illnesses. Curcumin has been shown in studies to be a powerful SIRT1 activator. Curcumin increases SIRT1 in a rat model of aging, encourages monoamine production, and then enhances cognitive performance [17]. Hippocampal-dependent spatial learning capacity is evaluated using the MWM learning test. It is one of the most extensively used methods to assess animal learning and memory [18].

It combines reference memory, working memory, and spatial learning, which are three independent memory functions. In addition, it is an unpleasant way of assessing hippocampus memory's cognitive performance. Prenatal cholinergic muscarinic antagonist injection reduces learning and memory, whereas inhibiting AChE increases cholinergic activity, which promotes this memory retention. AChE levels were shown to be correlated with learning capacity and cognitive ability in the water maze in the cholinergic system of the hippocampus. Data from several research point to a distinct role for hippocampus local GABAergic circuit neurons in the learning of spatial relationships. The current study's MWM test findings throughout the training period in all groups revealed typical learning profiles, as evidenced by falls in escape latencies from day 1 to day 4. A reduction in the day 4 EL of control group mice showed the normal memory acquisition, and an increase in the amount of time spent in the TQ looking for the disappeared platform at the retrieval trials demonstrated memory protection. Donepezil and curcumin treatment significantly lowered the escape latencies of mice with CYP-induced cognitive impairment, indicating that the curcumin enhanced the impaired long-term memory (reference memory) produced by CYP [19,20].

Animals' spatial long-term memory was inferred using the EPM paradigm (transfer latency). The animals are able to escape the risky open arm more quickly on the second trial because they can recall how the enclosed and open arms are set up. The test allows for the evaluation of the transfer latency procedure's fear-motivated learning component. In mice, shortened transfer latency on day 2 of the experiment is used as a metric for memory consolidation or retention, and drug treatment on day 1 may also be used to measure the acquisition-related effects of medicines. The current study recommends that combination of curcumin along with CYP for memory-enhancing activities because of their facilitative effects on spatial memory retention in CYP-induced cognitive impairment. Animals were instantly able to detect the dark zone after being put in the open arm in the EPM paradigm, indicating a decrease in the telomere length (TL) and an increase in cognition. The group treated with CYP displayed a substantial rise in TL values on both the acquisition and retention days, indicating impairment in learning and memory [21,22].

The MPA test was selected to demonstrate long-term memory ability. It was observed that the passive avoidance response test is a quick and simple way to identify changes in learning and memory. It is dependent on the hippocampus and amygdala and is based on learning with some object or instrument that produces fear. Animals treated with CYP decreased the SDL at the time of the retention trial, showing memory impairment. Curcumin at doses of 200 mg/kg enhances SDL and reverses the memory impairment brought on by CYP. It showed a considerable improvement in memory and reversed the damage that CYP treatment had done to memory. This implies that the animal retains memories of the shock that was provided during the learning phase and remains in the area that is shock-free. In order to reverse the CYP-induced cognitive impairment, it was observed that the administration of curcumin to the animals had a significant impact on the retention of memory [23].

Formation of ROSs due to oxidative damage plays a major role in the degeneration of neurons. The buildup of ROS in cells results in oxidative stress and neurodegeneration. One of the initial processes in the etiology of memory loss is oxidative stress. A well-known component that is crucial to maintaining a healthy equilibrium of oxidative stress is LPO. Short-term and long-term memory are impacted by oxidative stress. Through altering the structure of the synapse, such as by altering synaptic plasticity, memory loss may result. The main location for ROS synthesis is in the mitochondria, and failure in these structures causes an excess of ROS to be produced, which then causes adenosine triphosphate to be depleted and ultimately results in cell death. The electron transport chain's production of superoxide anion radicals during oxidative phosphorylation is the main source of ROS. In order to be detoxified by CAT and GSH peroxidases, superoxide ions must first be converted to H_2O_2 by the enzyme SOD [24].

In the brain, GSH plays a crucial intracellular scavenging role and is a component of the antioxidant defense system. When GSH peroxidases are present, GSH's catalytic thiol group is oxidized to form GSSG, which is then converted to GSH by the enzyme GSH reductase. This process is what gives GSH its antioxidant properties. GSH is converted to GSSG by GSH peroxidases, which then catalyzes the detoxification of H_2O_2 to water and oxygen molecules. CYP treatment reduces the activity of GSH after 21-day administration. Comparing the curcumin-treated group with the CYP treatment group, brain GSH levels significantly increased [25].

The enzyme SOD gets changed into superoxide radical anion to hydrogen peroxide (H_2O_2) by oxidative degradation. SOD activity is increased following treatment with donepezil and curcumin compared

to the CYP -treated group [26].

Reactive oxygen metabolites (ROMs), which induce neurotoxicity, are among the secondary products of LPO, which is regarded to be a harmful kind of oxidative deterioration that damages cell membranes. It has been established that a rise in MDA, one of the ROMs, is a key signal of stress produced by ROS. In this study, CYP-induced mice had higher MDA levels than healthy control mice. MDA significantly decreased in animals after receiving curcumin and donepezil in comparison to the mice treated with CYP alone [27].

A heme-containing tetrameric protein is being produced when the body is exposed to oxygen. Inside the cell, there is a spontaneous production of an antioxidant enzyme, CAT. Peroxidases catabolize H_2O_2, which is produced inside the cell through an enzymatic reaction. CAT is an effective enzyme for H_2O_2 saturation. Using iron (Fe) as a cofactor, it produces water, oxygen, and alcohol after interacting with H_2O_2.

Administration of the standard drug donepezil and test drug curcumin enhances the CAT level in comparison to the animals treated with CYP [28].

CONCLUSION

The results indicated that curcumin has a neuroprotective effect in experimental mice against CYP-induced cognitive impairment. In comparison to mice who received CYP treatments separately, those who received a 21-day course of curcumin therapy performed better cognitive activity. Therefore, the association of curcumin along with CYP in cancer chemotherapy may be a therapeutic option by which the adverse event of CYP, i.e., cognitive deficit, can be protected. In addition, this combination therapy improved CNS toxicity by recovering the altered level of SOD, CAT, GSH, and MDA induced by CYP in the mouse brain. These results provide a sound justification for the combined administration of curcumin, which may serve as an effective adjuvant therapy for memory impairments.

AUTHOR'S CONTRIBUTION

Ashutosh Kumar Yadav designed the pharmacological experiments on laboratory animals, collection of data, and finalized the manuscript. Dr. Harinath Dwivedi assisted in the experimental design and provided expertise in data collection and writing the discussion part. Dr. Dharamveer managed the statistical analysis and was involved in the preparation of figures and tables.

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CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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