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RESEARCH ARTICLE
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NEUROPROTECTIVE ROLE OF PHOSPHODIESTERASE-5 INHIBITOR IN CYCLOPHOSPHAMIDE-INDUCED COGNITIVE DEFICITS: BEHAVIORAL AND BIOCHEMICAL ASSESSMENT IN SWISS ALBINO MICE.

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Abstract:

Background: Chemotherapy-induced cognitive impairment, often referred to as "chemobrain," is a common and debilitating side effect of cancer treatment. Cyclophosphamide (CPA), a widely used chemotherapeutic agent, is known to induce oxidative stress and cognitive dysfunction. Phosphodiesterase-5 (PDE-5) inhibitors have shown neuroprotective potential by enhancing cGMP signaling, improving cerebral blood flow, and reducing oxidative damage. This study aimed to evaluate the neuroprotective effects of a PDE-5 inhibitor against CPA-induced cognitive deficits in Swiss albino mice.

Methods: Male Swiss albino mice (35–40 g) were divided into four groups (n = 6/group): Control, CPA (100 mg/kg, i.p., single dose), PDE-5 inhibitor only (5 mg/kg, p.o.), and CPA + PDE-5 inhibitor. The PDE-5 inhibitor was administered daily for 14 days. Cognitive function was assessed using the Y-maze and Novel Object Recognition (NOR) tests. Biochemical analyses of brain tissues included malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and acetylcholinesterase (AChE) activity.

Results: CPA administration significantly impaired spatial and recognition memory, increased MDA levels, and reduced GSH and SOD activity, along with elevated AChE activity. Treatment with the PDE-5 inhibitor significantly reversed these effects, improving behavioral performance and restoring oxidative and cholinergic balance.

Conclusion: PDE-5 inhibition demonstrated significant neuroprotective effects against CPA-induced cognitive dysfunction in mice, likely via antioxidant and cholinergic mechanisms. These findings support the potential therapeutic role of PDE-5 inhibitors in managing chemotherapy-related cognitive impairment.

Keywords: Cyclophosphamide, PDE-5 inhibitor, chemobrain, cognitive impairment, oxidative stress, acetylcholinesterase, Swiss albino mice.

Introduction:

Touchy mental need is a condition where someone emphatically conflicts with comprehension - their mental endpoints like memory or thinking. In MCI these difficulties are more awful than would continually be run of mill for a super person of their age. Regardless, unconstrained effects are insufficient insane to infringe according to a general viewpoint with commonplace presence, as are not portrayed as dementia.[1]

It is evaluated that a couple of spot in degree of 5 and 20 percent of people developed north of 65 have MCI. It's start & end close by a sort of dementia, yet a person with MCI will indeed go onto help dementia. This factsheet explains what MCI is affiliation between MCI, dementia & upsides of diagnosing MCI. It then looks at treatments for MCI ways to deal with administering acclimating to signs & how you can reduce your bet of making MCI & dementia. Many people not completely settled to have MCI use this as an entry to change their lifestyle to other than foster things.[2]

The term MCI portrays a lot of signs, rather than a specific affliction. A person with MCI everything considered despises something like one of going with:

- Memory-Forgetting consistent events or reiterating an overall arrangements.
- Thinking, figuring out or definitive speculation attracting with totally considering things.
- Thought being immediately upset.
- Language-taking far beyond anyone's expectations surprisingly lengthy to find right word for something.

Visual significance information endeavoring to decipher an article in three perspectives, judge kills or investigate stairs.[3]

Epidemiology of delicate mental impedance:

Since MCI powers its own fascinating achievement weight and gathers the bet of dementia, it is basic to ceaselessly analyze standards of MCI beginning with one side of the world then onto following. Regardless, uncovered confirmation of MCI everything considered shifts across studies & ranges some spot in degree of 3 & 54%. It is felt that this cutoff can be explained by division in research structure, significance in India.[4]

Clinical attestation of touchy mental deficiency:

Enormous thought should be given to course of action history. A few medications, including sedatives, narcotic, anticonvulsants or anti-cholinergics could perhaps sway sagacious cutoff. An exact neurological assessment is indispensable to pick likely etiology of mental impairment. For specific seeing it is incredibly crucial for talk with patient's relative or close frivolity, which knows about their functioning in progressively works out, requiring planning, association, & correspondence skills.[5]

Tadalafil:

Tadalafil is a fix used to treat erectile dysfunction (ED), innocuous prostatic hyperplasia (BPH), and pneumonic vein hypertension. It is a tablet taken by mouth. Starting is everything considered inside thirty minutes & compass relies on 36 hours. Common unavoidable outcomes strip cerebral torment, muscle torture, flushed skin, & nausea. Caution is reprimanded in those with cardiovascular disease. Stunning yet gigantic certain outcomes concrete a surrendered erection that can lead to dazzling quality to the penis, vision issues, and hearing event. Tadalafil isn't proposed in people taking nitro vasodilators, for instance, nitroglycerine, as this could achieve a solid drop in circulatory strain.

Cyclophosphamide:
Cyclophosphamide (CP), other than proposed ascytophosphane is a medication used as chemotherapy & to cover immune system. As chemotherapy it is used to treat lymphoma, multiple myeloma, leukemia, breast cancer, little cell breakdown in lungs, neuroblastoma & sarcoma. As a monitored silencer it is used in nephrotic condition, granulomatosis with polyangiitis and following organ move, among other conditions. It is taken by mouth or blend into a vein. Past what might be overall around expected most partner presumably results. Standard side effects include low white platelet counts, loss of yearning, tossing, going uncovered, and annihilating from bladder. Other genuine yielded results join a long future risk of issue, terribleness, upsettingly slight reactions, & pulmonary fibrosis. Cyclophosphamide is in the alkylating agent and nitrogen mustard get-together of approach.

Material and Methods: Study was conducted in the of department of Pharmacology and Therapeutics during a period from January 2023- December 2024 at Rama Medical college and associated hospital, Kanpur (U.P.).
The study was approved by the scientific review board and institutional ethics committee.

Experimental animals
Swiss albino mice weighing 35-40 gm will be used for the study. Total thirty rats are used & divided into 5 groups with each group having 6 rats. Animals will be adapted to multi week before inception of test & will kept under standard laboratory conditions like animals will be housed under controlled room temperature 23±2°C and allowed water & food (red pellet) in free access with 12hr light dark cycle and at relative humidity of approx. (50±15).

Equipments
• Spectrophotometer (UV Double beam)
• Ph meter
• Deep freezer
• Micro Centrifuge
• Micropipette
• Tissue homogenizer
• Electronic weighing balance

Chemical regent:
- Tadalafil, Piracetam
- Cyclophosphamide (CPA)
- Carboxymethylcellulose (0.5%)
- Phosphate Buffered Saline (PBS)
- Thiobarbituric Acid (TBA)
- Trichloroacetic Acid (TCA)
- Hematoxylin and Eosin (H&E)
- ELISA Kits for TNF-α, IL-1β
- NADPH

Type of study: it will be experimental study design.
Study duration: Research study will be duration of one year.
Sample size: n = 6 animals per group

Biochemical parameters
• Thiobarbituric acid reactive substance
• Reduced glutathione

• Super oxide dismutase (SOD)
• catalase (CAT)

Histopathological analysis
• Hematoxylin & eosin (H&E) staining
Neurobehavioral tests
• Y-maze
• Nort test

In vivo approach: Total sleep deprivation model
Swiss albino mice (weight- 35-40gm) and housed in polypropylene limits in an especially ventilated room (air cycle: 12-15 per hour; degree - 50:40) under an incorporating temperature of 23±2°C and 40-65% relative humidity, with a 12h light & 12h weak fake photoperiod. Creatures pushed toward feed and water. Mice were adjusted to a time of multi week to evaluation area going in advance the initiation of experiment.

Total sleep deprivation model
Total sleep deprivation (TSD) was induced by modified multiple platform technique. Briefly, small platforms (8.5 X 2.5 cm) were put (7 cm away from each other) inside the separated district containing water 2 cm under the stage surface. Mice had choice to truly move from one stage to another yet couldn't unwind across between stages to rest. water in tank was changed twofold every day. All mice had free assurance to direct & water. Mice were TSDfor 72 h.
Animals were divided into six groups with 10 in each. Tadalafil and piracetam were administered daily at 18:00- 18:30 h.

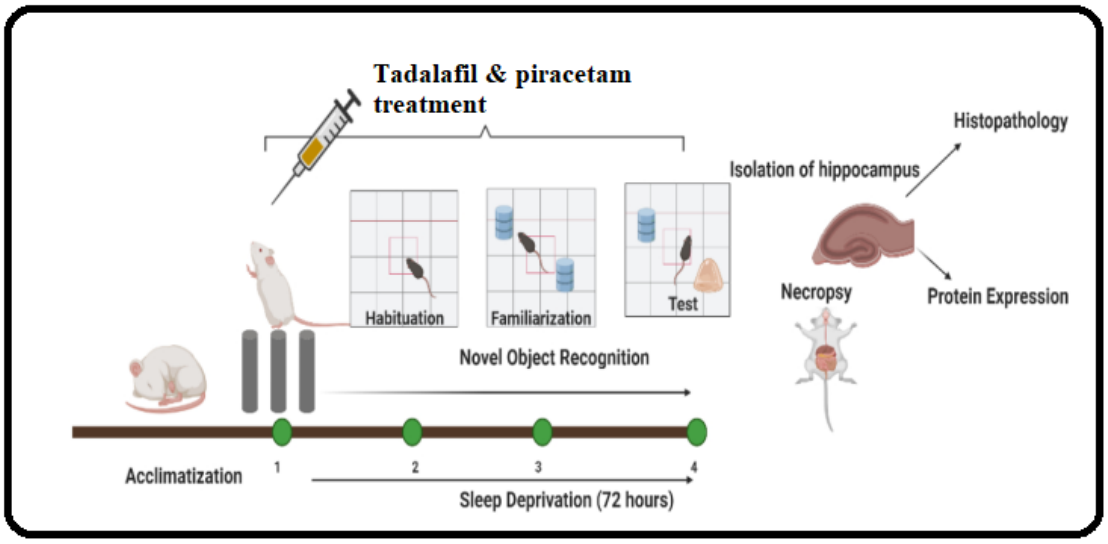


Figure:1. Experimental design of total sleep deprivation model in Swiss albino mice

Memory assessment
Novel object recognition test
Novel object recognition test (NORT) was done to determine the impact of TSD on mice. This test consists of three parts: -
(a) **Habituation:** Mice were put in the perplex box (36 × 50 × 36cm)for 10 minutes to acclimatize (2nd day of treatment).
(b) Training: Two similar objects were placed in a box at 10 cm away from each other. Individual mouse was introduced into the box to explore the objects for 10 minutes (3rd day of treatment).

(c) Assessment: Assessment was done on the 4th day. One object is swapped by a novel object. Mice that were left in the box were observed for 10 minutes. This whole assessment was video recorded and the object preference for the mice was noted down. Discrimination index was determined using the formula check formula, where parenthesis opens and closes
DI=RI/ (time spent in exploring novel object + time spent in exploring familiar object)
Recognition index= time spent in searching novel object – time spent in searching familiar object

Morris Water maze
The MWM test was done as per the method by with a minor modification to determine impact of chronic sleep restriction on spatial memory in mice. A circular drum (diameter: 125 cm; height: 36cm) filled with water was split equally in four quadrants. Skimmed milk was used to make the water turbid. Platform was placed in NW quadrant, 1 cm underneath H_2O. Mice were exposed to acquisition trial (exercise to find the hidden platform) twice in a day to 5 days. During trial phase mice were left in drum from SE quadrant around the edge of the drum. Mice were permitted to search for the hidden platform. If mice were unable to locate the platform within 90 s, they were directed towards the hidden platform. Probe test was done on the 6th day, platform was removed to test the retention memory of mice. Mice were allowed to swim in the drum for a period of 60s, assessment was video recorded and ANY-maze software was used to determine the escape latency, distance travelled, number of entries in the target quadrant and track plot of the mice.

Hippocampus isolation
Immediately after finishing the behavioral assessment animals were euthanized under anesthesia. Head and brain regions were removed. Upon removal, hippocampal regions were dissected out and immediately placed into tubes on dry ice. Samples were stored in -80^0C until used. For studies examining protein expression, hippocampal region of the mice was sonicated in truly cold radioimmuno-precipitation investigate cushion containing protease inhibitor mixed drink & centrifuged at 10000 rpm for 10 m at 4oC. Supernatant was collected and stored in -80^0C until used.

Total protein
Cells/ mice hippocampus were lysed with radioimmuno-precipitation assay buffer containing protease inhibitor. Total protein content was established by bicinchoninic acid protein assay. Lysate samples were aliquoted & stored at -80^0Cuntil used.

Histopathology
10% neutral buffered formalin was used as a fixing solution for mice brain. Brain tissues were embedded in paraffin and coronal locales (3-5 μm) of hippocampus region were cut using microtome. Region were mounted on a slide, washed, & got dried out with 95% ethanol and stained with cresyl violet, hematoxylin & eosin (H&E), Congo red for histopathological examination.

Data analysis
All the data values were expressed as Mean ± Standard deviation (SD). One-way analysis of variance followed by Tukey's multiple comparison test was used for statistical analysis. The significant criteria were fixed as $p \leq 0.05$.

Result:
Total sleep deprivation model Effects of tadalafil and piracetam on recognition memory in total sleep deprived mice
Impact of TSD on recognition memory and novelty preference was assessed using NORT. In the familiarisation period, all the experimental animals spent equal time in similar objects. During recognition memory test (24h after familiarisation), the vehicle treated TSD mice spent equal amount of time in the familiar and novel object while the vehicle saline spent significantly more time in exploring the novel object.

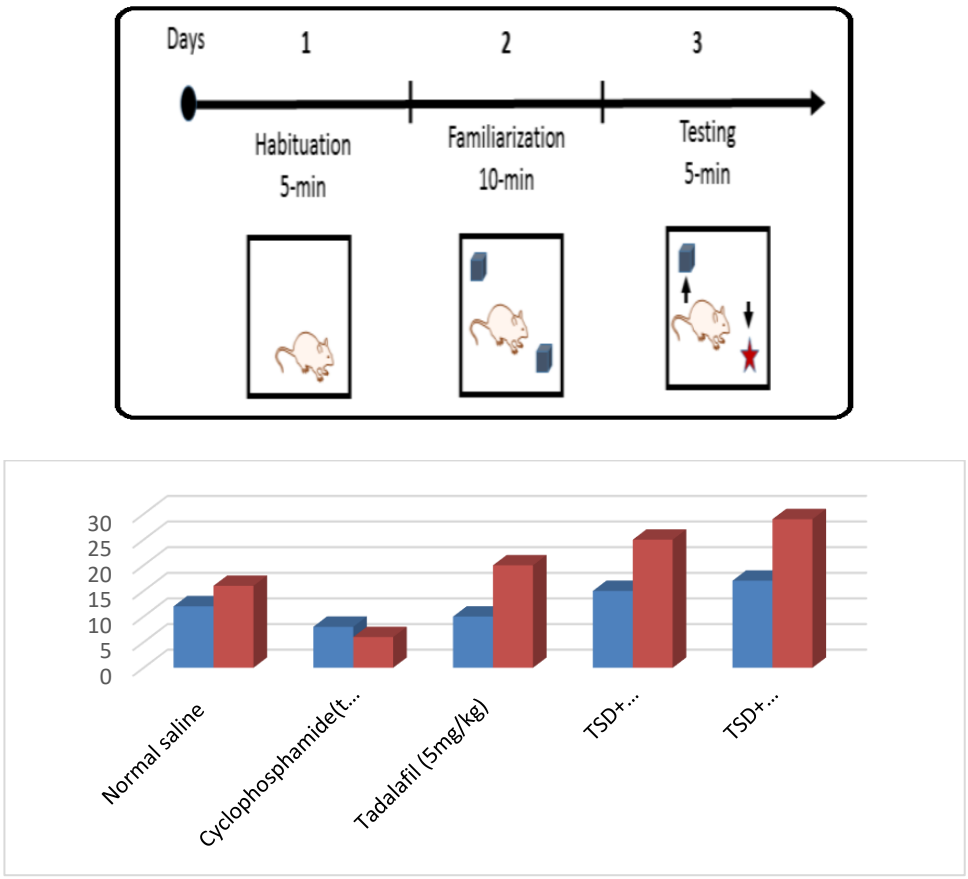


Figure 2. Tadalafil and piracetam administration restored TSD induced cognitive dysfunction in mice.

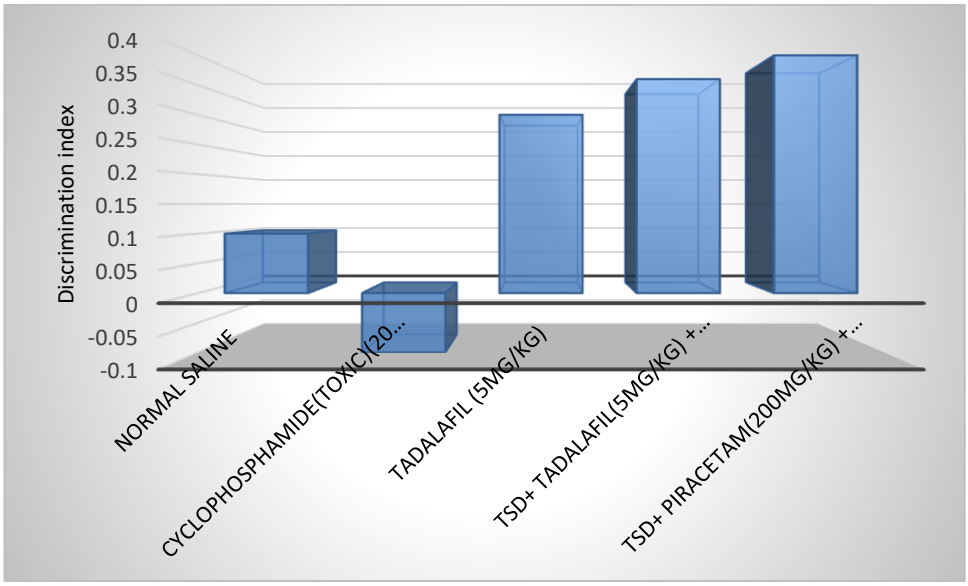
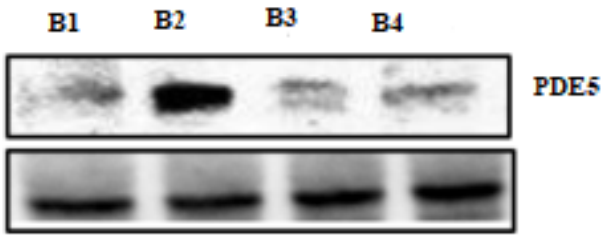


Figure 3. Tadalafil and piracetam administration improved the discrimination index between the objects in TSD mice.

Effect of TSD on PDE5 expression in hippocampal mice

Total sleep deprivation increased the hippocampal PDE5 expression in vehicle treated mice. Administration of tadalafil produced dose dependant downregulation of PDE5 expression in TSD mice when compared to the vehicle treated TSD mice. A significant decrease was observed at

tadalafil(5mg/kg). Similarly, vehicle treated TSD mice also showed upregulation of PDE5 expression in TSD mice. Piracetam administration reduced PDE5 expression in TSD mice.



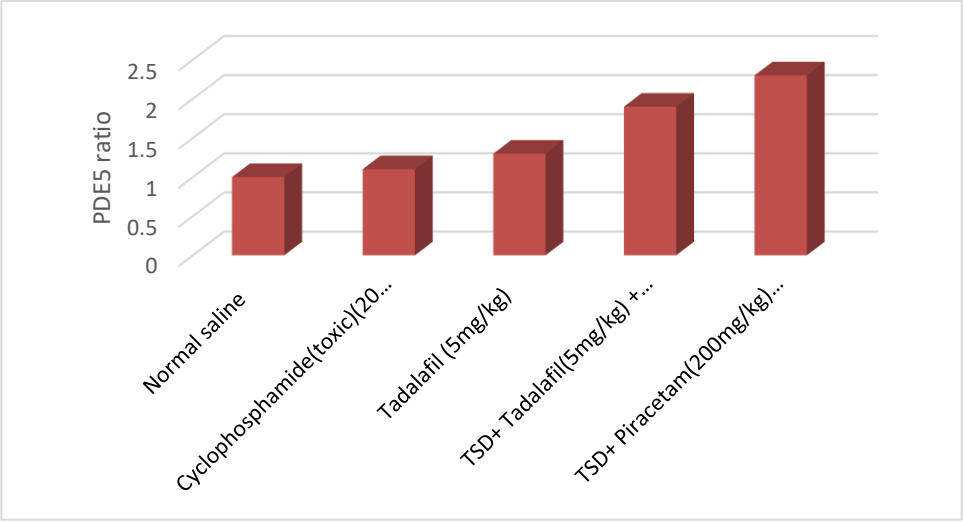


Figure 4. Tadalafil and piracetam administration reduced PDE5 expression in TSD mice.

Effect of TSD on Aβ deposition in hippocampus region of mice

Preclinical and clinical studies have shown that increases Aβ deposition in hippocampusand this causes cognitive impairment. Tadalafil and piracetam treated TSD mice showed a significant decrease in the expression of Aβ comparedwith vehicle treated TSD group.

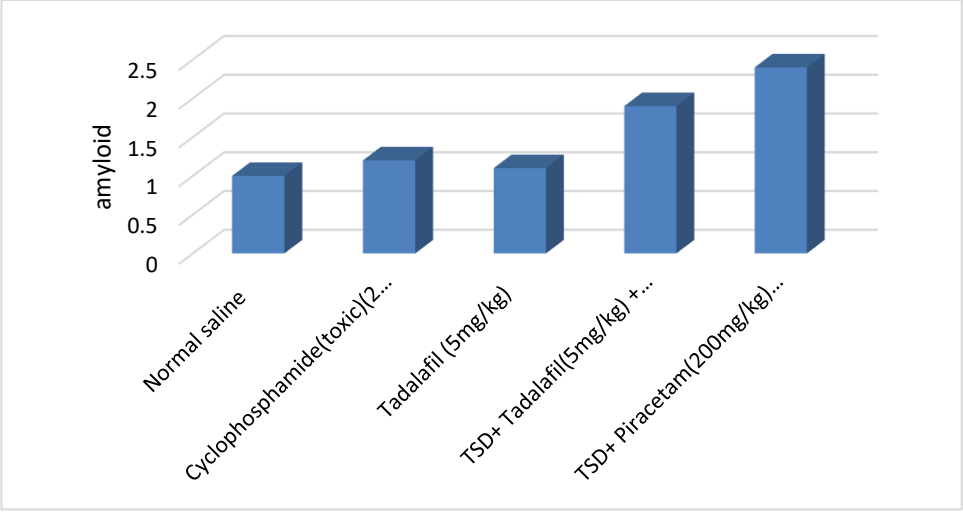


Figure 5. Tadalafil and piracetam administration reduced TSD induced Aβ expression in mice.

This was further confirmed with Congo red staining in the hippocampus region in TSD mice. We found that vehicle treated TSD mice showed intense staining indicating accumulation of Aβat CA1 and DG regions of hippocampus. Tadalafil and piracetam treated TSD mice showed to reduce the deposition of Aβ at hippocampusCA1 and DG region when compared with vehicle treated TSD mice indicating PDE5 can prevent Aβ deposition in TSD mice.

Biochemical Estimation
Superoxide Dismutase Activity

Table:1 Effect of tadalafil on SOD, CATALASE, TBARS, and GSH content in hippocampus

Treatment Mg/kg	SOD (u/mg of wet tissue)	CAT (u/mg of wet tissue)	GSH (u/mg of wet tissue)	LPO (u/mg of wet tissue)
Vehicle control	20.56 ±1.12	14.68± 1.74	16.4 ±4.48	26.82 ±3.10
CP 200(toxic)	6.76 ±1.67	6.71± 1.79	23.93 ±1.12	7.81± 1.56
TD as per	16.18 ±1.13	15.75±0.83	16.31 ±1.37	28.89± 1.80
TD 5mg + CP 200	8.88±1.57	9.84±2.00	13.44± 2.08	11.36± 1.67
Piracetam 200 + CP 200	13.95±1.59	14.69±1.68	15.67±1.67	26.80***±1.43

ALL values are expressed as mean ± SEM (n=6).

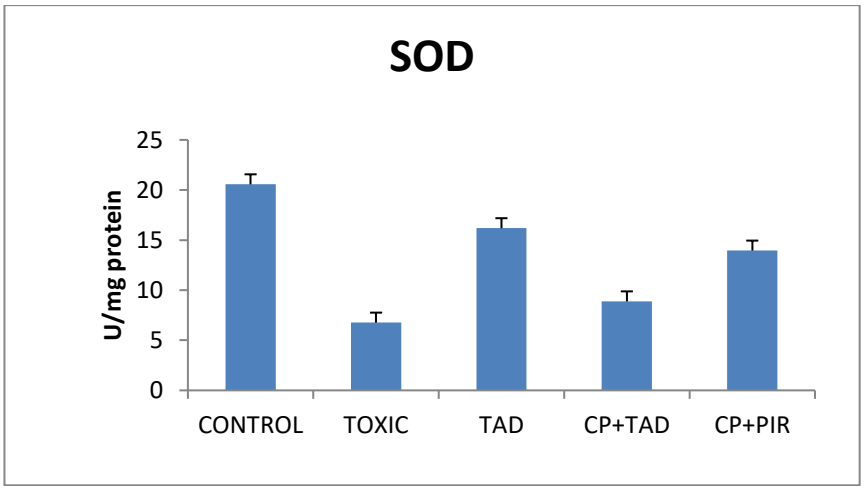


Figure:6 Data of SOD (superoxide dismutase)

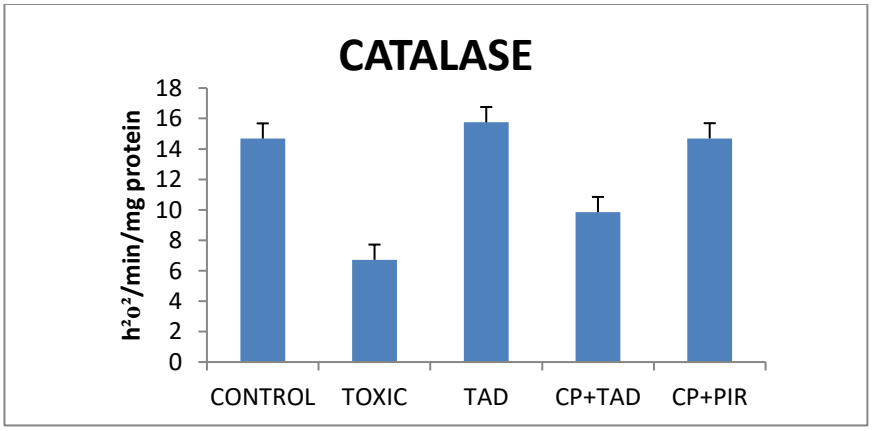


Figure: 7 Data of Catalase

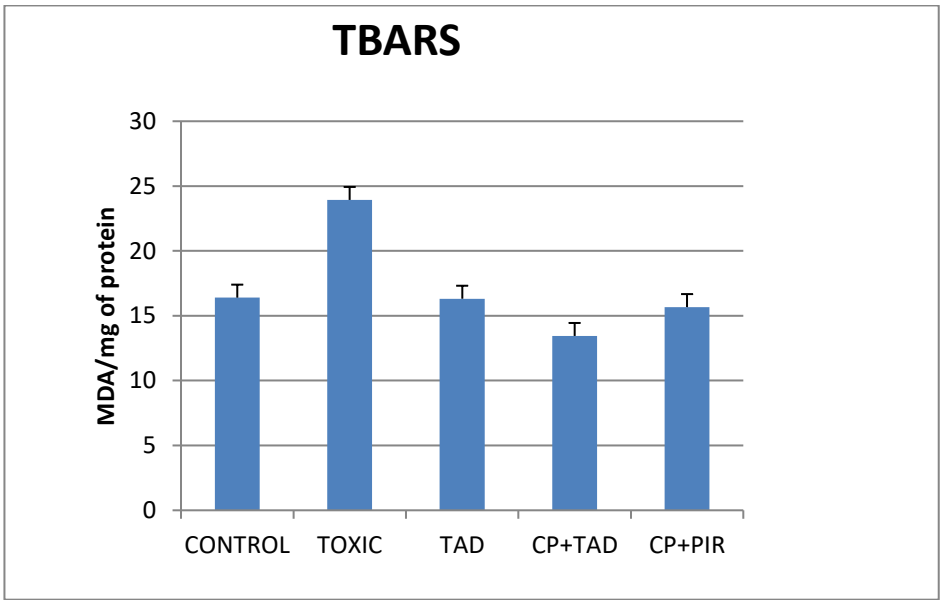


Figure: 8 Data of TBARS

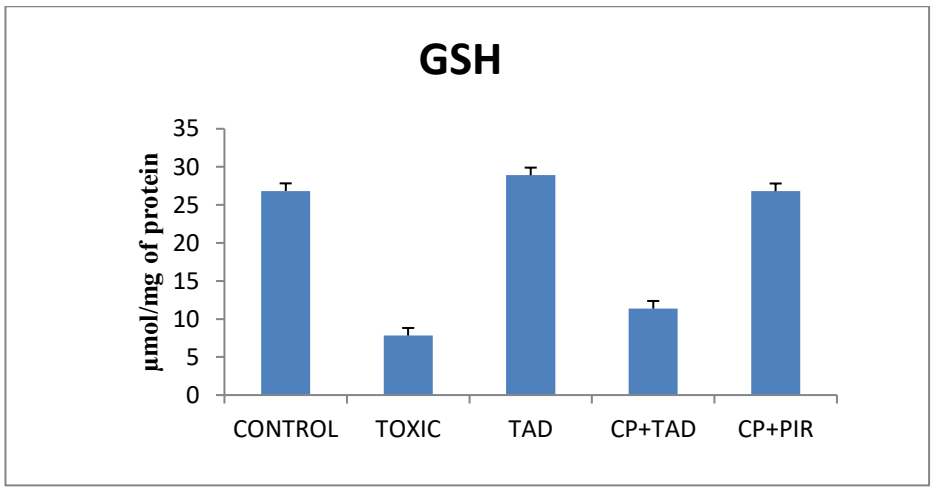


Figure: 9 Data of GSH

Discussion: The present study investigated the neuroprotective potential of a phosphodiesterase-5 (PDE-5) inhibitor against cyclophosphamide (CPA)-induced cognitive deficits in Swiss albino mice. The findings from behavioral and biochemical assessments suggest that PDE-5 inhibition may ameliorate chemotherapy-induced cognitive impairment.

PDE-5 inhibitors, such as sildenafil and tadalafil, increase intracellular cyclic guanosine monophosphate (cGMP) levels by inhibiting its degradation. These effects may be attributed to restoration of cholinergic neurotransmission, reduction in oxidative stress, and potential anti-inflammatory properties. [6]

Biochemical analyses supported the behavioral findings. PDE-5 inhibitor treatment significantly reduced MDA levels and restored SOD and GSH levels, indicating attenuation of oxidative stress. Additionally, AChE activity was normalized in the CPA + PDE-5 group, suggesting improved cholinergic tone, which is critical for memory formation.

these results align with previous studies that have reported the neuroprotective and cognitive-enhancing effects of PDE-5 inhibitors in various models of neurodegeneration, including Alzheimer's disease, vascular dementia, and stroke. While most existing literature focuses on age-related or amyloid-induced cognitive decline, our study adds to the growing body of evidence that PDE-5 inhibition may also be effective in mitigating chemotherapy-induced cognitive dysfunction.[7]

Conclusion: TSD/CSR increases the protein expression of PDE5 and Aβ deposition in hippocampal region and down-regulates synaptic protein expression in mice brains, which could be the possible underlying pathogenic mechanism. The present study evidence that restoration of NMDA receptor activity, autophagy process and cAMP cascade by PDE5 inhibitors can prevent the cognitive decline induced by sleep deprivation or chronic sleep restriction. Interestingly, the investigated small molecules tadalafil and piracetam reversed the pathological, molecular changes and improved cognitive produced without any notable adverse effects. The study warrants further investigation on the interaction between Aβ and NMDA receptors and the direct role of PDE5 inhibition on the said targets. These findings will help to reposition the use of PDE5 inhibitor in subjects vulnerable to sleep alterations, to restore cognitive functions and induced neurodegeneration.

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