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RESEARCH ARTICLE

Protective Effect of Edaravone on Cyclophosphamide Induced Oxidative Stress and Neurotoxicity in Rats

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ARTICLE HISTORY

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Abstract: ***Background*:** Cyclophosphamide (CPA) is the most widely prescribed cancer chemotherapeutic agent which shows serious neurotoxic side effect. Generation of reactive oxygen species at the cellular level is the basic mechanism of cyclophosphamide induced neurotoxicity. Edaravone is the synthetic drug used for brain stroke and has potent antioxidant property.

***Objective*:** This study aimed to investigate the effect of edaravone on neurobehavioral and neuropathological alteration induced by cyclophosphamide in male rats.

***Methods*:** Twenty eight Sprague-Dawley rats were equally divided into four groups of seven rats in each. The control group received saline, and other groups were given CPA intraperitoneally (100 mg/kg), CPA (100 mg/kg) intraperitoneally + Edaravone (10 mg/kg) orally, or Edaravone (10 mg/kg) orally for one month.

***Results*:** Our data showed that CPA significantly elevated brain AChE activity in the hippocampal region. A decrease in the total antioxidant capacity and a reduction in the CAT, SOD, and GPX activity occurred in the brains of the rats exposed to CPA. CPA-treated rats showed a significant impairment in long-term-memory and motor coordination. These results were supported by histopathological observations of the brain. Results revealed that administration of edaravone reversed AChE activity alternation and ameliorated behavioral and histopathological changes induced by CPA.

***Conclusion*:** This study suggests that co-administration of edaravone with cyclophosphamide may be a useful intriguing therapeutic approach to overcome cyclophosphamide induced neurotoxicity.

Keywords: Cyclophosphamide, edaravone, neurotoxicity, oxidative stress, neuroprotective, AChE.

1. INTRODUCTION

Cyclophosphamide (CPA) is a cancer chemotherapy agent generally utilized in cancer management to enhance the life expectancy of malignant growth patient. Despite a restorative impact, CPA is additionally connected to numerous unfortunate symptoms because of its metabolites, phosphoramide mustard and acrolein [1]. Other side effects include nephrotoxicity, hepatotoxicity, urotoxicity, cardiotoxicity, immunotoxicity, mutagenicity, genotoxicity, carcinogenicity, teratogenicity, and neuronal toxicity [2-5]. The CPA metabolite acrolein builds lipid peroxidation and delivers exceptionally reactive oxygen species (ROS). These overabundant ROS interface with different cells and cause cellular impairments [6, 7]. The neurotoxicity of cyclophosphamide and its metabolites has been well established [8-11]. By lessening the abundance of ROS, it is conceivable to limit the toxicity related to cyclophosphamide.

Antioxidant compounds have been shown to shield tissues from cyclophosphamide-induced oxidative impairment [12]. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a free extreme scavenger that is as of now utilized in the treatment of acute ischemic stroke as a neuroprotective reagent, has been shown to essentially diminish the infarct size, enhance neurological scores, and decline ROS age [13]. More explicitly, it can balance toxicity from enacted microglia [14]. Neuroinflammation in Middle Cerebral Artery Occlusion (MCAO) might be weakened by edaravone which acts through concealment of the expression of proinflammatory cytokines in enacted microglia [15].

2. MATERIALS AND METHODS

2.1. Chemicals

All essential chemicals were purchased from Sigma-Aldrich, India. MDA, SOD activity, GSH level, and GPx activity assay kits were purchased from IBL International.

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2.2. Animals

Grown-up male Sprague-Dawley rodents (5 weeks old, 180g) were obtained from Animal House, Jawaharlal Nehru Cancer Hospital and Research Center, Bhopal. Rodents were housed in plastic pens in an animal room with a temperature of 22 ± 2 ºC and humidity of 55 ± 5% and 12h light: dark cycle. The animals got nourishment (Altromin 1324) and tap water was not obligatory. The examinations followed the rules of the Institutional Animal Ethical Committee ((IAEC/Truba/Proj/05).

2.3. Experimental Design

In this examination, rodents were arbitrarily partitioned into four gatherings (n = 7 for each gathering). The first gathering animals were administered intraperitoneally with 0.9% saline once every week for about a month. The second gathering animals were administered intraperitoneally with CPA at 100 mg/kg once per week for about a month. The third gathering animals were administered intra peritoneally CPA at 100 mg/kg once every week for about a month with edaravone at 10 mg/kg orally, once daily for about a month. The fourth gathering animals were administered edaravone at 10 mg/kg, orally, once daily for about a month. After all medications at about a month, the behavioral tests were done and after that, samples were gathered. The animals were habituated to the pimpairmentacology lab for one hour before beginning the test.

2.4. Behavioral Tests

2.4.1. Passive Avoidance Test

A detached evasion test was performed to assess the impacts of SA or EA on the long-term memory in rodents. The investigations were carried out utilizing a step-through sort detached evasion apparatus (PACS-30, Columbus Instrument, USA), comprised of equivalent estimated light and dark compartments (22×21×22cm), and isolated by a guillotine entryway. A 40W light was fixed 30cm over the floor at the focal point of the light compartment. The floor was produced using stainless steel and associated with a stun trigger. Single electrical stuns (0.5mA, 75V, 50Hz) were conveyed to the matrix floor of the dark compartment by a trigger. Before the behavioral screening, the animal was prepared for the instrument. In the instructional meeting, each rodent was set in the lit up compartment and permitted 1min for habituation. The guillotine entryway was opened and shut promptly when the animal entered the dark compartment; at that point, an electric stun was conveyed through the matrix floor. In the test session, every animal was again set in the enlightened compartment, 24h after the instructional meeting. The progression through inertness to enter the dark compartment was estimated in the two sessions. The cut-off time was 600s instructional course [16].

2.4.2. Rotarod Test

Rotarod test is done by utilizing the rotarod gear (Rotamex, Columbus Instrument, USA) for screening the motor execution of animals. The unit comprises a pivoting axle, a power hotspot for turning the axle and lattices underneath the turning roller where the rodent can fall without damage. All animals were pre-prepared on the rotarod device so as to achieve a steady execution. Three separate preliminaries were incorporated into the animal trail on day 1, with somewhere around one hour of rest between preliminaries, under a quickening convention beginning at 4rpm and achieving 40 rpm in 5 min and the dormancy to fall was recorded [17].

2.4.3. Sample Collection

Rodents from various gatherings were executed by beheading after the finish of the behavioral test. Brain tissues were isolated and then washed with saline quickly. For histological studies, a part of cortex was fixed in 10% phosphate buffered formalin. For biochemical and AChE activity estimations, the hippocampus of the brain was homogenized (1/10 w/v) in ice-cold Tris-HCl buffer (0.1M, pH7.4).

2.4.4. Biochemical Measurements

Hippocampi from various gatherings were homogenized for estimation of the antioxidant status of the mind [18]. In short, one gram of mind tissue was gauged and homogenized in 0.1 M chilled potassium phosphate support (PH 7.4) utilizing Denville Ultra EZ grind tissue homogenizer for 5 min. The homogenate was centrifuged at 14000 rpm at 4 °C for 15 min to acquire supernatant that was utilized to assess the centralization of malondialdehyde-lipid peroxidation marker-(MDA) [19], and decrease glutathione (GSH) [20] with catalase (CAT) [21], superoxide dismutase (SOD) [22] and glutathione peroxidase (GPX) [23] exercises at Shimadzu spectrophotometer (UV120-02).

2.4.5. Measurement of AChE Activity

To gauge AChE action of the hippocampus, the tissues were homogenized in 10 volumes of a super cold (medium I), comprising 320 mM sucrose, 0.1 mM ethylenediamine tetraacetic acid, and 5 mM 4-(2-hydroxyethyl)- 1-piperazine ethanesulfonic acid, pH 7.5 in (TissueRuptor, Qiagen) homogenizer and Synaptosomes were confined utilizing an intermittent Percoll inclination [24]. At that point, the pellet was suspended in an isoosmotic arrangement, and the last protein focus was acclimated to 0.5 mg/mL. Synaptosomes were arranged new day by day, kept up at 4 °C all through the technique, and utilized for enzymatic examines. The response blend (2 mL last volume) contained 100 mM K+ - phosphate cushion, pH 7.5 and 1 mM 5,5′-dithio-bis-nitrobenzoic acid. The strategy depends on the arrangement of the yellow anion, 5,5′-dithio-bis-acid nitrobenzoic, estimated by perusing absorbance at 412 nm amid a 2-min brooding at 25 °C. The protein was pre-incubated for 2 min, and the response started by including 0.8 mM acetylthiocholine iodide (AcSCh). All examples were kept running in copy or triplicate and catalyst action was communicated in μmol AcSCh/h/mg of protein [25].

2.4.6. Histopathological Examination

Tissue tests were settled in 10% unbiased formalin for 24h and paraffin squares were obtained and routinely handled for light microscopy. Cuts of 4-5 μm were acquired from the readied squares and recolored with H and E. The arrangements obtained were envisioned utilizing Omano microscopy (OM150-MK 40X-400X) at amplification of ×400.

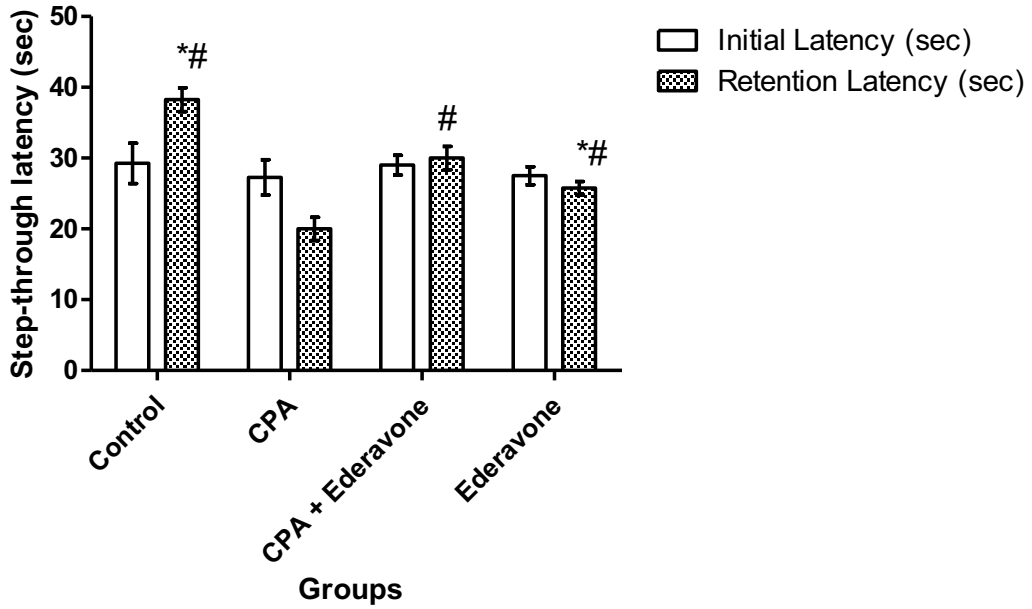

Fig. (1). Cyclophosphamide effect on long-term memory impairment in passive avoidance test. Values are expressed as mean +SB (N=7). Data were analyzes by two-way ANOVA followed by Bonferroni post-test. *p <0.05 significant difference retention latency as compared to the initial latency same groups, #p <0.05 significant difference as compared to CPA-treated group.

2.4.7. Statistical Analysis

Information examination was performed with GraphPad Prism programming (form 5). All the outcomes were communicated as mean ± SD. Every measurable correlation was made by methods for one-way ANOVA test pursued by Tukey's post hoc investigation and p-esteem < 0.05 was viewed as huge.

3. RESULTS

3.1. Behavioral Tests

3.1.1. Effect of Edaravone on Learning and Memory Impairment Induced by Cyclophosphamide

As shown in Fig. (**1**), organization of cyclophosphamide fundamentally diminished the progression through inactivity when contrasted with the control gathering (p<0.05), while the multi-week organization of edaravone altogether turned around the memory weakness incited by cyclophosphamide (p< 0.05).

3.1.2. Effect of Edaravone on Motor Coordination Impairment Induced by Cyclophosphamide

The result of the rotarod analysis demonstrates that organization of cyclophosphamide diminished the falling inactivity when contrasted with the control gathering (p<0.001). Besides, the multi-week edaravone organization essentially expanded the dormancy to fall initiated by cyclophosphamide (p<0.001) (Fig. **2**).

3.2. Biochemical Measurements

3.2.1. Effect of Edaravone on Cyclophosphamide-induced Oxidative Imbalance in the Brain

There is unevenness induced in the middle of oxidative and antioxidant parameters in brain homogenate after cyclophosphamide organization. Oxidative unevenness was shown by a factually huge (p< 0.05) increment in hippocampus MDA focus with a noteworthy decline in GSH dimension of rodents after cyclophosphamide when contrasted with control rodents (Table **1**). Additionally, the brain exercises of CAT, SOD, and GPX estimated a critical (p< 0.05) decline in cyclophosphamide-treated rodents. Normalizing these oxidative changes following the multi-week organization of edaravone in cyclophosphamide-treated rodents affirmed the cell reinforcement job of edaravone in the hippocampus.

3.3. AChE Activity

3.3.1. Effects of Cyclophosphamide on AChE Activity in the Hippocampal Homogenates

In the control gathering, AChE activity was 4.41 μmol AcSCh/h/mg proteins in the hippocampal homogenates. Organization of cyclophosphamide indicated increased AChE action essentially 56.4% as a contrast with vehicle-treated gathering. Multi-week continuous edaravone treatment fundamentally lessened the AChE activity (Fig. **3**).

3.4. Histopathological Study

Minuscule examination of the cortex demonstrated that cyclophosphamide caused degeneration in the most analyzed districts portrayed by the nearness of necrotic and apoptotic cores (Fig. **4**).

4. DISCUSSION

This examination was completed to research the impact of edaravone treatment on cyclophosphamide and uncovered rodents' hippocampus oxidative status, AChE action, and conduct modification. The discoveries of present examinations are in consonance with prior investigations, where researchers announced that memory and learning conduct is represented by the hippocampal district of the mind and

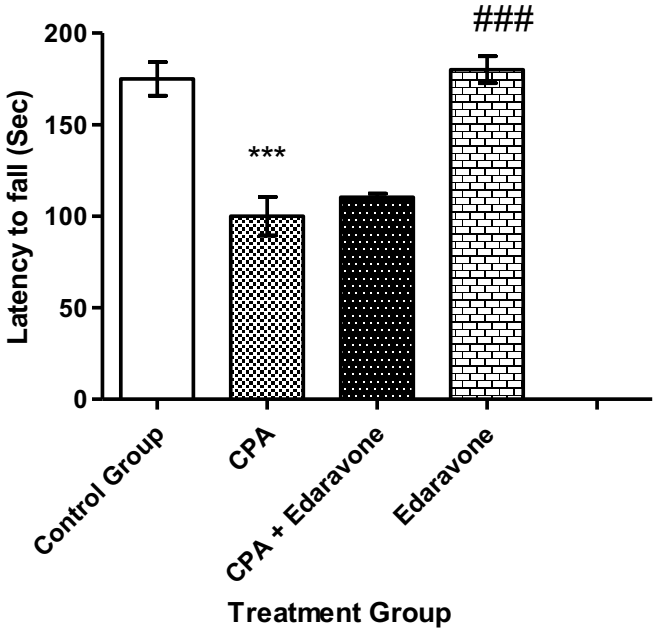

Fig. (2). Effect of edaravone treatment on latency to fall in CPA-induced motor coordination and equilibrium impairment. Values are means + SD (n=7). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. significant difference in comparison with the control group (**p < 0.001). #significant difference in comparison with the CPA-treated group (###p < 0.001).

Table 1. Effect of cyclophosphamide on oxidative status of brain with the role of edaravone.

Treatment	Control Group	CPA Treated Group	CPA + Edaravone Treated Group	Edaravone Treated Group
GPX (µmol NADPH/g tissue)	$10.97 \pm 0.54^{\text{¥\$}}$	$14.89 \pm 0.78^{\text{¥}}$	$4.23 \pm 1.67^{\#}$	$9.96 \pm 0.94^{\$}$
MDA (µmol/g tissue)	$32.12 \pm 1.33^{\#}$	$27.98 \pm 0.98^{\$}$	$47.97 \pm 1.87^{\text{¥}}$	$33.45 \pm 0.97^{\#}$
SOD (U/g tissue)	$20.98 \pm 1.34^{\$}$	$29.23 \pm 1.32^{\text{¥}}$	$11.89 \pm 0.78^{\#}$	$21.24 \pm 1.22^{\$}$
GSH (mg/g tissue)	$18.94 \pm 0.98^{\$}$	$28.09 \pm 1.23^{\text{¥}}$	$13.45 \pm 0.98^{\#}$	$20.12 \pm 1.97^{\$}$
CAT (µmol H_2O_2 decomposed/g tissue)	$52.02 \pm 1.32^{\$}$	$59.89 \pm 1.87^{\text{¥}}$	$37.23 \pm 2.12^{\#}$	$52.89 \pm 0.96^{\$}$

Values are expressed as mean ± SD for each group. $^{\#, \text{¥}, \$}$ are the means within the same column and bearing different superscripts are significantly different at $p < 0.05$.

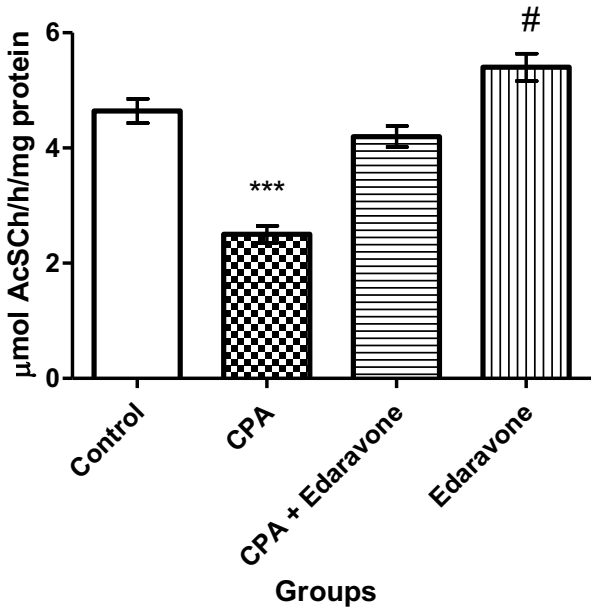

Fig. (3). Acetylcholinesterase (AChE) activity in hippocampal synaptosomes. Values are means + SD (n=7). Data were analyzed by one-way ANOVA followed by turkey's post hoc test multiple comparisons. significant difference in comparison with the contro; group (***p < 0.001). #significant difference in comparison with the CP A-treated group (#p < 0.04).

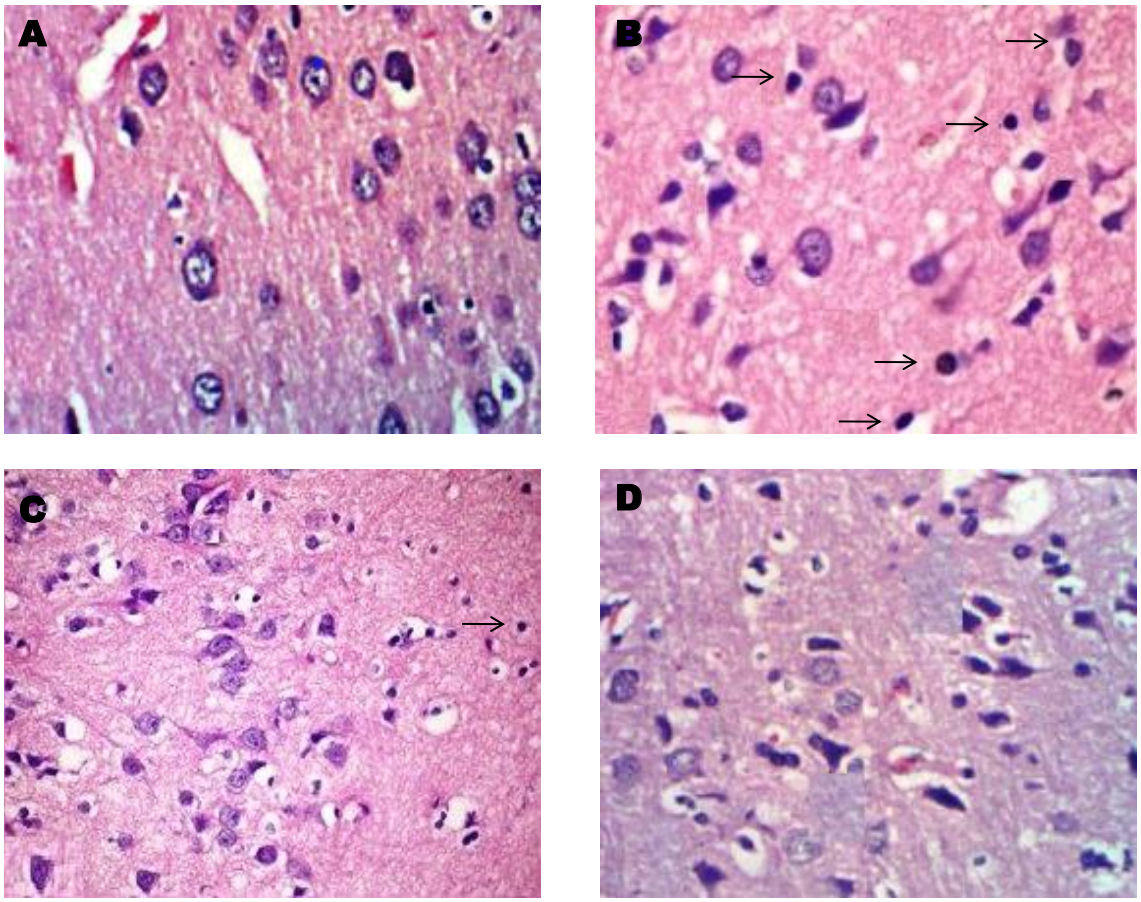

Fig. (4). Histopathological lessions of brain tissue. **A**) Control group showing normal histological features of cortex with well-formed neurons. **B**) CPA group showing cyclophosphamide (CPA) treated rats with damaged cortex. **C**) CPA + Edaravone group showed with better recovery in tissues and well-formed nuclei without vacuolation and irregular features. **D**) Edaravone group showing edaravone treated animal brain with normal tissues as control group H&E 20 mm.

cyclophosphamide incites hippocampal toxicity [26-29]. The decline in the action dimension of AChE in the mind area exhibited in this investigation demonstrates a decrease of cholinergic neural connections in the cyclophosphamide uncovered hippocampus rodents. A recent report identified with cyclophosphamide neurodegenerative conduct likewise shows the inhibitory capacity of cyclophosphamide on a cholinergic framework [30, 31]. Thus, an adjustment in conduct parameters, for example, memory weakness and motor coordination in cyclophosphamide uncovered rodents can be the consequence of concealment of the cholinergic framework by cyclophosphamide.

A few investigations have demonstrated that oxidative stress is a significant component of cyclophosphamide incited neurotoxicity. In the hippocampus, it actuates impairment to mitochondria and causes mitochondrial brokenness, bringing about unnecessary ROS age [32]. This component is specifically connected with cell oxidative stress, which impairs the antioxidant resistance framework and results in cell passing and neuronal impairment [33]. Additionally, different reports estimated the endogenous antioxidant proteins and a few oxidative stress markers to assess the dimension of oxidative worry in cyclophosphamide uncovered animals, and prompts over the top oxidative stress and causes cell toxicity [34, 35].

By considering oxidative worry as a fundamental component of cyclophosphamide actuated neurotoxicity, we assessed edaravone as a neuroprotective remedial specialist. Edaravone is principally utilized as the neuroprotective specialist in different neurodegenerative conditions related to over the top oxidative stress age [36]. Edaravone fills in as an antioxidative specialist and avoids lipid peroxidation [37]. Also, edaravone indicates a defensive job on isoproterenol-induced myocardial dead tissue in rodents where oxidative stress and aggravation assume the pivotal job in ailment activity [38]. Furthermore, the ongoing investigation uncovered that edaravone enhanced the cholinergic framework, shielded neurons from oxidative toxicity and improved spatial learning and memory shortfalls in the rodents [39].

The dimension of lipid peroxidation in the rodent mind was assessed in this examination by estimating MDA level in the rodent hippocampus. MDA is one of the real pointers of lipid peroxidation since it is created towards the finish of lipid peroxidation. Lipid peroxidation is straightforwardly connected with over the top oxidative stress, in this way estimating MDA level is an often utilized technique [40, 41]. In this investigation, edaravone improved cyclophosphamide initiated increment of MDA level, proposing that edaravone treatment diminished the dimension of oxidative stress. With MDA in this investigation, we additionally assessed the

SOD, GPx exercises and GSH levels as these catalysts and synthetic compounds are straightforwardly related to cell reinforcement framework. SOD is one of the vital antioxidant enzymes in the host guard framework against oxidative stress. Superoxide (O^{-}_{2}) is one of the real ROS that causes oxidative stress on different cells and organs, and SOD changes it to sub-atomic oxygen and less poisonous hydrogen peroxide (H_2O_2), bringing about the diminished oxidative impairment in the brain. It is commonly realized that the dimensions of SOD and GPx exercise increment with expanding oxidative stress cells. Nonetheless, according to announced examinations, it tends to be reasoned that antioxidant proteins, for example, SOD and GPx could be impaired and diminished by exorbitant oxidative stress, bringing about a decline of cell reinforcement protection [42, 43]. GPx is likewise imperative in cell reinforcement framework. It is an essential cell reinforcement protein since it changes H_2O_2 to water (H_2O). The dimension of GPx diminished with expanded oxidative worry in cells [44].

GSH is known to be a vital cofactor of GPx-intervened redox response. Low dimensions of GPx and GSH have corresponded with oxidative stress related conditions [45]. In this examination, cyclophosphamide introduction induced reductions in SOD, GPx, and GSH levels in the rodent hippocampus when contrasted with the control gathering and edaravone kept these cyclophosphamide actuated declines in antioxidant catalysts and related synthetic concoctions in check. Consequences of the above investigation show that the defensive impact of edaravone could be credited to improve cyclophosphamide incited reductions in SOD, GPx, and GSH levels. This defensive impact of edaravone can be because of its property as cell reinforcement that searches free radicals and keeps antioxidant catalysts from extreme oxidative impairment in the brain [46]. The atomic instrument of edaravone is to repress both water solvent and lipid dissolvable peroxyl radical-induced peroxidation framework. Its belongings are, in this manner, like the consolidated impacts of nutrients C and E. Edaravone represses both nonenzymatic lipid peroxidation and lipoxygenase pathway through its electron giving properties and has powerful antioxidant impacts against mind hoisted oxidative stress conditions [47].

We have, in our investigation, additionally shown that cyclophosphamide presentation instigates impedance in conduct and diminishes AChE activity which was additionally enhanced by edaravone treatment. Expanded AChE action levels mirror the expanded acetylcholine discharge which would encourage the synaptic transmission of neurons. The upgrade of subjective capacities by edaravone treatment ascribed to its capacity to improve mind AChE activity. An ongoing report has announced that the ameliorative impact of edaravone against psychological brokenness intervened through the decrease of AChE level [39]. As indicated by past investigations, edaravone has a solid helpful potential to secure neurons [48], glia (microglia, astrocytes, and oligodendrocytes) [49-51], and vascular endothelial cells [52] against oxidative stress and has been shown to stifle the incendiary reaction of enacted microglial cells [53]. Ongoing findings appreciated that edaravone demonstrates defensive activity against STZ-induced intellectual impedance, oxidative stress, cholinergic brokenness and changed protein expressions in streptozotocin incited psychological hindrance in rodents [54]. It is likewise detailed that edaravone improves learning and memory and advances the development of neurons [55].

All in all, it may very well be determined from the present outcome that edaravone offers huge neuroprotective potential against cyclophosphamide-induced neurotoxicity in exploratory rodents. The introduction of cyclophosphamide caused motor incoordination, learning and memory shortfalls, and expanded AChE and oxidative worry in the hippocampus. Treatment of edaravone produces an ameliorative impact on rodents by expanding motor activity, intellectual capacity, and AChE action and decreasing oxidative stress. Consequently, this examination expresses the use of edaravone amid cyclophosphamide chemotherapy which may viably keep the chemotherapy-instigated psychological deficiencies.

CONCLUSION

It can be concluded by this study that edaravone can be therapeutically beneficial for overwhelming the toxic effect of cyclophosphamide by improving the brain antioxidant status and normalizing brain neurotransmitter level.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This project has been approved by the Institutional Animal Ethics Committee (Regn. No. CPCSEA- MMCP/IAEC/15/15), at Truba Institute of Pharmacy, Mullana, Ambala, India.

HUMAN AND ANIMAL RIGHTS

No humans were used in the study. All the reported experiments on animals were in accordance with the recommendations of the Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forests and Environment, Government of India.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author (S.S) upon reasonable request.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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