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GABA MODULATION ON ADRENALECTOMY INDUCED DEMENTIA IN WISTER RAT

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Abstract

This study elucidates the neuroprotective potential of GABAergic modulation in a unilateral adrenalectomy (ADX)-induced rat model of dementia. Surgical ablation of the adrenal glands disrupts corticosteroid homeostasis, precipitating oxidative stress, hippocampal neurodegeneration, and marked cognitive deficits. Gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the central nervous system, was explored for its therapeutic efficacy in mitigating these neuropathological changes. GABA administration significantly ameliorated spatial learning and memory impairments, ostensibly through the attenuation of excitotoxicity and stabilization of synaptic transmission. Furthermore, biochemical analyses revealed a restoration of hippocampal glutathione levels, underscoring an enhancement in endogenous antioxidant defense mechanisms. These findings underscore the potential of GABA receptor modulators as promising candidates in the management of stress-induced cognitive dysfunction associated with adrenal insufficiency**.**

Keywords Neuroprotective, Adrenalectomy, Gamma-aminobutyric acid, Antioxidant

Introduction

Dementia encompasses a group of disorders that impair cognitive, behavioral, emotional, and social functions, leading to significant disability and increased morbidity and mortality. The most common forms include Alzheimer's disease (AD), vascular dementia (VaD), frontotemporal dementia, semantic dementia, and dementia with Lewy bodies. AD and VaD together account for approximately 85–90% of all dementia cases. Globally, dementia represents one of the most pressing public health challenges, with approximately six million new diagnoses annually. Due to diagnostic delays, many individuals remain undiagnosed during the early stages despite evident cognitive deficits [1]. Mild cognitive impairment (MCI) is considered a prodromal phase, with increased risk of progression to dementia [2]

Clinically, dementia is characterized by progressive cognitive decline severe enough to interfere with social and occupational functioning. Symptoms typically include deterioration in memory, reasoning, daily functioning, and behavioral abnormalities [3]). While dementia predominantly affects individuals over the age of 65, early-onset cases have also been reported [4].

Age-related hippocampal dysfunction is a well-established phenomenon in both animal models and humans, rendering neurons more vulnerable to neuropathological insults and injury. With advancing age, cumulative physiological stress contributes to sustained activation of the hypothalamic–pituitary–adrenal (HPA) axis, a process implicated in the pathogenesis of numerous neurodegenerative diseases (NDDs), including Alzheimer's disease (AD) [5].

Although stress is a universal aspect of daily life and generally well-managed, chronic exposure to significant stressors can disrupt physiological homeostasis, ultimately leading to behavioral and cognitive disturbances [6]. According to the concept of allostatic load, persistent internal or external stressors can surpass adaptive capacity, resulting in biological changes that predispose individuals to disease [7]. Dementia has been strongly associated with an overproduction of reactive oxygen species (ROS) and free radicals. NADPH oxidase (NOX), a key enzyme complex responsible for ROS generation, has emerged as a central contributor to oxidative stress in the brain. NOX catalyzes the reduction of oxygen

using NADPH to produce superoxide anions, promoting oxidative damage in neuronal tissues [8]. Other enzymatic sources of ROS include nitric oxide synthase, cyclooxygenase, lipoxygenase, cytochrome P450, and xanthine oxidase. NOX-mediated activation of microglia leads to both extracellular and intracellular ROS production, exacerbating neuroinflammation [9].

ROS derived from cerebrovascular cells further impair endothelial function and microcirculatory processes in the brain, contributing to cellular dysfunction, apoptosis, and neurodegeneration [10].

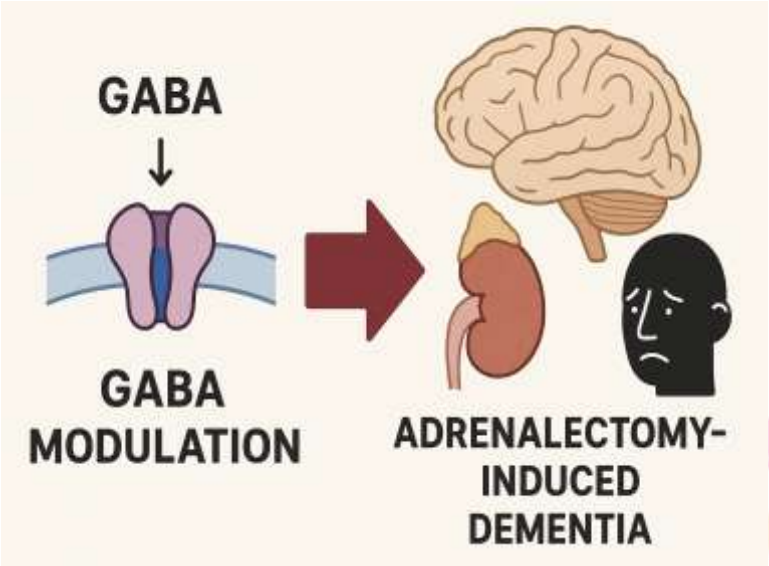

Figure – 1 GABA modulation on adrenalectomy induced dementia effect in human

MATERIALS AND METHODOLOGY

Chemical and reagents

All the chemicals and reagents were used in analytical grade and purchased from scientific supplier Roorkee and Vikash scientific work Roorkee Haridwar UK.

Animals

The animals on which the current investigation was performed, of Albino Wistar rats weighing between 180 and 250g (Registration No. 1147/ab/07/CPCSEA)

Unilateral adrenalectomy

Unilateral adrenalectomy was performed via a dorsal approach under ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia, administered intraperitoneally. A small incision was made on the left dorsal side to expose and excise the left adrenal gland. The incision was closed with sutures, and animals received gentamicin (35 mg/kg, i.p.) for 5 days post-surgery to prevent infection.

Experimental groups.

Table -1 Experimental protocol of Adrenalectomy induced Dementia in Wister Rat

Experimental Protocol																
Treatment				Cold Stress (4°C for 1 hr)			Elevated Plus Maze			Morris Water-Maze						
S. No.	Groups						TL			ELT				TSTQ		
							Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Control						Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8		
2	Normal Saline					Day 1	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14		
3	Sham UA					Day 1	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14		
4	Topiramate Per se D1					Day 1	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14		
5	Topiramate Per se D2					Day 1	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14		
6	Unilateral Adrenalectomy (UA)	Day 1	Day 10	Day 11	Day 15	Day16	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29		
7	UA + Topiramate (10mg/kg)	Day 1	Day 10	Day 11	Day 15	Day16	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29		
8	UA + Topiramate (25mg/kg)	Day 1	Day 10	Day 11	Day 15	Day16	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29		
9	UA + Donepezil (10mg/kg)	Day 1	Day 10	Day 11	Day 15	Day16	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29		
		No Treatment					Drug/Vehicle Treatment									

Assessed Body weight

a weighing balance, the animal's body weight was determined on the first and end days of the research (Electronic). Any change in the body weight were tracked and the degree of significance was evaluated using statistical analysis utilising Sigma State software

Muscle Co-ordination activity

The rota-rod test was performed. The rod was used to rotate each animal from 3 to 30 rpm over a period of 3 minutes. A rotarod is then observed to see how long the animal maintains its position over it. The duration of the task is directly proportional to the animal's ability to maintain balance, which is dependent on brain and motor coordination. Two consecutive trials with a 180-second maximum cutoff time were performed on the animals[11]

Evaluation of Locomotor activity

Actophotometers are a commonly used instrument for measuring animal movement, were used to measure their locomotion. The basal activity scores of individual animals were obtained over a 5-minute period using actophotometers. After 30 minutes post-administration, animal activity levels were measured after receiving the appropriate treatment [12,13]

Assessment of variation in weight of different Organs

Rats will be sacrificed and the liver, kidneys, heart and brain will be isolated and will be weighed in relation to their body weight [14]

BEHAVIOURAL PARAMETERS

Elevated Plus Maze (EPM) Test

Spatial learning and memory were assessed using the Elevated Plus Maze (EPM). The apparatus consisted of two open arms (49 × 10 cm) and two closed arms (49 × 10 × 49 cm) arranged on a central platform (10 × 10 cm), elevated 65 cm above the ground under dim lighting. Each rat was placed at the center and allowed to explore for 10 minutes. Time spent in the open arms (TSOA) was recorded as an index of memory. Day 1 TSOA indicated acquisition, Day 2 reflected retention, and Day 3 was considered a measure of memory consolidation.[15]

Morris Water Maze (MWM) Test

Spatial learning and memory were assessed using the Morris Water Maze. The test was conducted in a circular pool (diameter: 120 cm) filled with opaque water maintained at 22 ± 1°C. A hidden platform (10 cm diameter) was submerged 1–2 cm below the surface in a fixed quadrant. During the acquisition phase (Days 1–4), animals underwent four trials per day from different start points, each lasting a maximum of 60 seconds. Escape latency and swim path were recorded. On Day 5 (probe trial), the platform was removed, and the time spent in the target quadrant and number of platform crossings were measured to evaluate memory retention.[16]

BIOCHEMICAL ESTIMATION

Collection of blood sample

The rats cervical collapse euthanasia, blood samples were obtained via the retro-orbital route. After being at room temperature for 30 minutes, the serum was extracted from the blood by centrifuging it while fifteen minutes at 4000 RPM. Serum cholesterol and serum glucose levels were measured using the serum. [17]

Estimation of blood glucose

The blood sugar levels in blood samples may be determined using a variety of techniques. We employed a glucose kit (GOD/POD technique) in this investigation. The glucose concentrations in serum and plasma are assessed in vitro using the GOD/POD technique. Tinder's approach is the basis for glucose kit (GOD/POD) procedure, which uses enzymes such as glucose oxidase and peroxidase, chromogen-4-amino antipyrine, phenol, and other compounds. The process is simple, quick, and only requires one step. It is not affected by the haemoglobin, reducing drugs or other factors.[18]

Estimation of Cholesterol

Using a readily available commercial kit, the serum cholesterol levels were determined Spectro-photometrically at 340 nm using the cholesterol oxidase (CHOD-PAP) technique. .[19]

Estimation of Reduced Brain Glutathione (GSH)

Reduced glutathione (GSH) levels were estimated by mixing equal volumes of serum or tissue supernatant with 10% trichloroacetic acid, followed by centrifugation at 1000 rpm for 10 minutes at 4°C. The supernatant (0.5 mL) was mixed with 2 mL of 0.3 M disodium hydrogen phosphate and 0.25 mL of freshly prepared DTNB (1% w/v in sodium citrate). After incubation for 20 minutes, the yellow colour formed was measured at 412 nm using a UV spectrophotometer (Shimadzu UV-1800). GSH concentration was determined from a standard curve and expressed as µM GSH/mg protein.[20]

Evaluation of Superoxide dismutase (SOD) Activity

SOD activity was measured using the Beauchamp and Fridovich method with a UV spectrophotometer (Shimadzu UV-1800). The reaction mixture contained 0.5 mL tissue homogenate, 1 mL of 50 mM sodium carbonate buffer, 0.4 mL of 24 µM NBT, 0.2 mL of 0.1 mM EDTA, and 0.4 mL of 1 mM hydroxylamine hydrochloride. The reaction was initiated by adding hydroxylamine, and the formation of a blue-coloured formazan was monitored at 560 nm. Absorbance was recorded every 30 seconds for 5 minutes at 25 °C. A blank without homogenate was used as control. [21]

Evaluation of brain catalase (CAT) activity

Catalase activity in brain homogenates was estimated spectrophotometrically by measuring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. The reaction mixture consisted of phosphate buffer (50 mM, pH 7.0) and freshly prepared 30 mM H_2O_2. Enzyme activity was expressed as µmol of H_2O_2 decomposed per minute per mg of protein**.** **[**22]

Assessment of Myeloperoxidase (MPO) Levels

MPO activity was determined in brain tissue homogenates using a spectrophotometric assay based on the oxidation of o-dianisidine dihydrochloride in the presence of hydrogen peroxide. The change in absorbance was measured at 460 nm, and enzyme activity was expressed as units per mg of protein.[23]

Estimate Serum TBARS levels

Serum thiobarbituric acid reactive substances (TBARS) levels were measured to assess lipid peroxidation. The assay is based on the formation of a pink chromogen from the reaction between malondialdehyde (MDA) and thiobarbituric acid (TBA), which was quantified spectrophotometrically at 532 nm. Results were expressed as nmol MDA/mL of serum**.** [24]

Assessment of Brain Acetylcholinesterase (AChE) activity

AChE activity was measured in brain homogenates using Ellman's method. The assay is based on the hydrolysis of acetylthiocholine iodide, producing a yellow-colored compound upon reaction with DTNB, measured at 412 nm. Enzyme activity was expressed as µmol of substrate hydrolyzed per minute per mg of protein. [25]

Histopathological Analysis

An examination of H&E staining shown a well-organized nerve cells arrangement in the control group's hippocampus regions

Statical analysis

All data were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used for multiple group comparisons. A p-value < 0.005 was considered statistically significant. Endothelial relaxation responses were analyzed using repeated measures ANOVA followed by Newman–Keuls test and expressed as a percentage of pre-contraction. Body weight and Morris Water Maze performance were evaluated using two-way ANOVA followed by Tukey's post hoc test**.**

RESULT

Effect of drug treatment on body weight

Unilateral adrenalectomy (UA) significantly reduced final body weight compared to control and sham groups. Treatment with Topiramate (10 and 25 mg/kg) and Donepezil (10 mg/kg) in UA animals showed a slight improvement in body weight, indicating partial protection against UA-induced weight loss.

Effect of muscle co-ordination activity

Unilateral adrenalectomy (UA) significantly reduced motor coordination time compared to control and sham groups. Treatment with Topiramate (10 and 25 mg/kg) and Donepezil (10 mg/kg) in UA animals showed a slight improvement in motor coordination activity, indicating partial recovery of muscle coordination.

Effect of locomotor activity

Unilateral adrenalectomy (UA) caused a slight reduction in locomotor activity compared to control and sham groups. Treatment with Topiramate (10 and 25 mg/kg) and Donepezil (10 mg/kg) in UA animals showed minimal improvement, indicating limited effect on restoring locomotor activity under these conditions.

Effect of drug treatment on organ weights in UA animals

The weights of different organs (heart, liver, kidney, and brain) across treatment groups are presented in Figure 3.4. Unilateral adrenalectomy (UA) led to a reduction in liver and kidney weights compared to control and sham groups. Treatment with Topiramate (10 and 25 mg/kg) and Donepezil (10 mg/kg) in UA animals showed a slight improvement in liver and kidney weights, suggesting partial protection against UA-induced organ weight reduction. No significant changes were observed in heart and brain weights across all groups**.**

Effect of drug treatments on transfer latency in UA animals

Transfer latency (TL) on the elevated plus maze is shown in Figure 3.5. Unilateral adrenalectomy (UA) significantly increased TL on the first day (TL1) compared to the control group ($p < 0.001$), indicating impaired learning and memory. On the second day (TL2), the UA group continued to show higher TL compared to controls, reflecting poor retention. Treatment with Topiramate (10 and 25 mg/kg) and Donepezil (10 mg/kg) in UA animals significantly reduced TL on the second day, indicating improvement in memory retention. Among the treatment groups, the UA + Donepezil group showed the most marked reduction in TL2, followed by UA + T2, suggesting effective memory enhancement in UA-induced cognitive impairment**.**

Effect of drug treatments on escape latency time in UA animals

Escape latency time (ELT) in the Morris water maze is shown in Figure 3.6a. Unilateral adrenalectomy (UA) animals exhibited a significant increase in ELT on day 4 compared to control animals ($p < 0.05$), indicating impaired spatial learning and memory. Control and sham groups showed a significant reduction in ELT on day 4 compared to day 1, reflecting normal learning ability. Treatment with Topiramate (10 and 25 mg/kg) and Donepezil (10 mg/kg) significantly reduced ELT on day 4 in UA animals ($p < 0.05$ vs. UA), indicating improvement in learning and memory performance. The UA + Donepezil group showed the maximum reduction in ELT, followed by UA + T2, suggesting a protective effect against UA-induced cognitive deficits**.**

Effect of drug treatments on time spent in the target quadrant in UA animals

Time spent in the target quadrant (TSTQ) on day 5 in the Morris water maze is shown in Figure 3.6b. Unilateral adrenalectomy (UA) animals showed a significant reduction in TSTQ compared to the control group, indicating impaired spatial memory retention. Control and sham groups exhibited higher time in the target quadrant, reflecting intact memory performance. Treatment with Topiramate (10 and 25 mg/kg) and Donepezil (10 mg/kg) significantly increased TSTQ in UA animals compared to the UA group, indicating improvement in memory retention. Among treatments, the UA + Donepezil group showed the highest increase in TSTQ, followed by UA + T2, suggesting enhanced cognitive performance in UA-induced memory impairment.

Effect of drug treatments on serum glucose levels in UA animals

Figure 3.7 shows the serum glucose levels across different experimental groups. Unilateral adrenalectomy (UA) induced a significant increase in serum glucose levels compared to the control and sham groups, indicating that adrenal insufficiency may lead to disturbances in glucose homeostasis. Treatment with Topiramate at both 10 mg/kg (T1) and 25 mg/kg (T2), as well as Donepezil (10 mg/kg), significantly reduced the elevated glucose levels in UA animals. Among the treatment groups, the higher dose of Topiramate (T2) and Donepezil exhibited a more pronounced reduction in serum glucose, suggesting their potential role in ameliorating hyperglycemia associated with unilateral adrenalectomy. These findings suggest that pharmacological intervention with Topiramate and Donepezil may help restore glucose homeostasis in conditions of adrenal insufficiency, potentially through neuroendocrine modulation and improved metabolic control**.**

Effect of drug treatments on brain GSH levels in UA animals

Figure 3.9 demonstrates the levels of reduced glutathione (GSH) in the brains of different groups. Unilateral adrenalectomy (UA) caused a significant reduction in brain GSH levels compared to control and sham groups, indicating increased oxidative stress and reduced antioxidant capacity due to adrenal insufficiency. Treatment with Topiramate (10 mg/kg and 25 mg/kg) and Donepezil (10 mg/kg) significantly increased brain GSH levels in UA animals compared to the untreated UA group. Among the treatment groups, the higher dose of Topiramate (25 mg/kg) and Donepezil showed better restoration of GSH levels, suggesting their potential antioxidant and neuroprotective effects in counteracting UA-induced oxidative stress. These findings indicate that Topiramate and Donepezil can mitigate oxidative stress in the brain by enhancing endogenous antioxidant levels, thereby providing neuroprotection in conditions of adrenalectomy-induced oxidative imbalance

Effect of drug treatments on serum cholesterol levels in UA animals

Figure 3.8 shows that unilateral adrenalectomy (UA) significantly increased serum cholesterol levels compared to control and sham groups, indicating dyslipidemia due to adrenal insufficiency. Treatment with Topiramate (10 and 25 mg/kg) and Donepezil (10 mg/kg) significantly reduced cholesterol levels in UA animals, suggesting their potential in ameliorating UA-induced hypercholesterolemia**.**

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Effect of of Superoxide dismutase (SOD) Activity

The superoxide dismutase (SOD) activity was significantly affected by the unilateral adrenalectomy (UA) and subsequent pharmacological treatments. As shown in Figure 3.10, the UA group exhibited a marked reduction in brain SOD levels compared to the control group, indicating oxidative stress induced by adrenalectomy. This was statistically significant and represented the lowest SOD activity among all groups. However, treatment with Topiramate at both low (T1, 10 mg/kg) and high doses (T2, 25 mg/kg) significantly improved SOD activity compared to the UA group. Among these, the combination group UA + T2 showed a more pronounced elevation in SOD activity, comparable to the control. Similarly, the standard treatment group UA + DNP (Donepezil, 10 mg/kg) also demonstrated a significant increase in SOD levels compared to the UA group. No significant differences were observed between the Control, Vehicle, Sham, and Peg-treated groups, suggesting that neither the vehicle nor procedural interventions influenced baseline SOD activity. These findings confirm that UA-induced oxidative stress, as evidenced by reduced SOD activity, is ameliorated by Topiramate and Donepezil treatment, highlighting their potential neuroprotective roles through antioxidant mechanisms.

Effect of brain catalase (CAT) activity

The catalase (CAT) activity in brain tissue was significantly altered following unilateral adrenalectomy (UA), The UA group showed a pronounced decrease in CAT activity compared to the control group, indicating impaired antioxidant defense and elevated oxidative stress. This group had the lowest CAT activity among all the experimental conditions. Treatment with Topiramate at both 10 mg/kg (T1) and 25 mg/kg (T2) significantly restored CAT activity compared to the UA group, with the higher dose (T2) showing a more substantial effect. The UA + T2 group demonstrated CAT levels approaching those seen in control animals. Similarly, the UA + DNP (Donepezil, 10 mg/kg) group showed a significant improvement in CAT activity over the untreated UA group. The Control, Vehicle, Sham, and PEG-treated groups maintained similar CAT activity, showing no statistically significant differences, indicating that neither the vehicle nor surgical procedures independently affected catalase expression. Overall, these findings suggest that UA induces significant oxidative stress by reducing catalase levels, which is effectively counteracted by Topiramate and Donepezil, affirming their antioxidant potential in neuroprotective therapy**.**

Effect of TBARS levels

Lipid peroxidation, assessed via TBARS levels, was significantly elevated in the UA group compared to control, indicating heightened oxidative stress (Figure 3.12). This group exhibited the highest TBARS concentration, marking severe lipid membrane damage. Treatment with Topiramate (both T1 and T2) and Donepezil (DNP) significantly

reduced TBARS levels, with UA + T2 and UA + DNP groups showing near-control levels, suggesting strong antioxidative effects. Control, Vehicle, Sham, and PEG-treated groups showed no significant changes. These findings indicate that UA induces oxidative lipid damage, which is effectively mitigated by Topiramate and Donepezil, demonstrating their neuroprotective potential.

Effect of Myeloperoxidase (MPO) Levels

Myeloperoxidase (MPO) levels were significantly increased in the UA group compared to control ($p<0.001$), indicating enhanced neuroinflammation (Figure 3.13). The elevated MPO activity reflects increased oxidative and inflammatory stress in the brain post-adrenalectomy. Treatment with Topiramate (T1 and T2) and Donepezil (DNP) significantly reduced MPO levels ($p<0.001$ vs. UA), with the UA + T2 and UA + DNP groups showing near-control values, indicating strong anti-inflammatory effects. No significant changes were observed in the Control, Vehicle, Sham, and PEG-treated groups. These results suggest that Topiramate and Donepezil effectively counteract UA-induced neuroinflammation.

Effect of Acetylcholinesterase (AChE) activity

The effect of unilateral adrenalectomy (UA) and subsequent pharmacological interventions on brain acetylcholinesterase (AChE) activity is presented in Figure 3.14. A significant elevation in AChE activity was observed in the UA group as compared to the control group, indicating enhanced cholinergic breakdown and impaired neurotransmission, which are hallmarks of cognitive dysfunction. Treatment with Topiramate at both doses—T1 (10 mg/kg) and T2 (25 mg/kg)—resulted in a statistically significant reduction in AChE activity compared to the UA group, suggesting restoration of cholinergic tone. Notably, the UA + DNP group (Donepezil 10 mg/kg), serving as the standard reference, also showed significantly reduced AChE activity, bringing levels closer to normal. The Control, Vehicle, Sham, and Per se groups (T1 and T2) exhibited comparable AChE levels, indicating no influence of procedural or drug-alone effects on basal AChE activity. These findings demonstrate that UA induces hyperactivity of AChE in the brain, which can be effectively mitigated by Topiramate and Donepezil, underlining their therapeutic potential in preserving cholinergic function post-adrenalectomy.

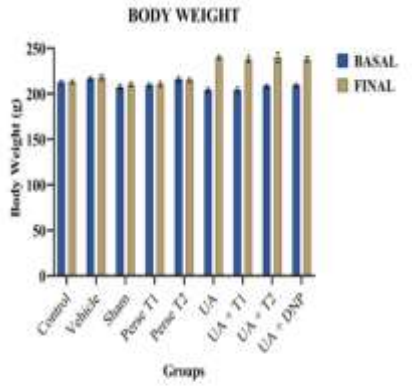

Fig 3.1: Effect of drug treatment on body weight in Unilateral Adrenalectomy (UA) animals: Mean ± SD, n=6; one-way ANOVA followed by Bonferroni's test; UA-Unilateral Adrenalectomy; T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

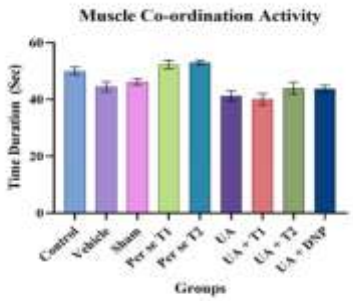

Fig 3.2: Effect of drug treatment on Motor Coordination activity in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Bonferroni's post-test; T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

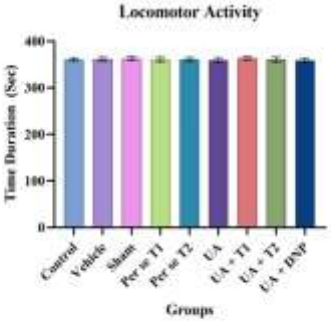

Fig 3.3: Effect of drug treatment on Locomotor activity in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Bonferroni's post-test; T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

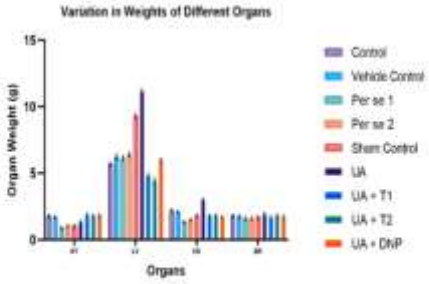

Fig 3.4: Effect of drug treatment on Organ weight in all animals: Mean ± SD, n=6; one-way ANOVA followed by Bonferroni's test; T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

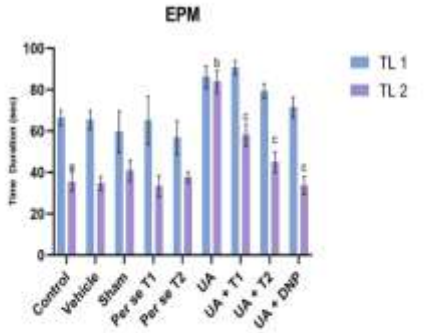

Fig 3.5: Effect of drug treatments on Transfer Latency (TL) in UA animals: Mean ± SD, n=6; Mean ± SD, n=6; one-way ANOVA followed by Tukey's test; F (8, 45) = 10.47; a p<.001 versus TL of previous days within each group; b p<0.001 versus TL of respective day in control group; c p<0.001 versus TL of respective day in UA animals. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

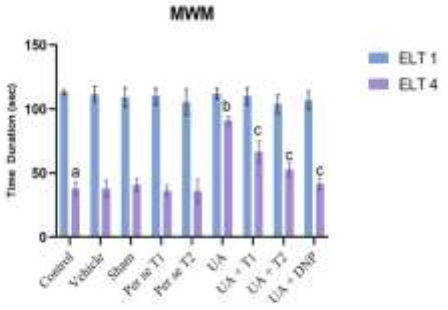

Fig 3.6a: Effect of drug treatments on Day 4 escape latency time (ELT) in Unilaterally Adrenalectomized animals: Mean ± SD, n=6; two-way ANOVA followed by Tukey's test; F (8, 45) = 34.16; a p<0.05 versus day1 ELT of control animal; b p<0.05 versus day 4 ELT of control animal; c p<0.05 versus day 4 ELT in UA animals. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

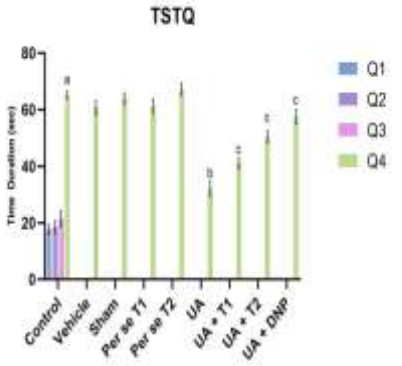

Fig 3.6b: Effect of drug treatments on Day 5 TSTQ in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Bonferroni's post-test; F (8, 45) = 174.7; a p<0.05 versus day 5 mean time spent in other quadrant; b p<0.05 versus day 5 TSTQ of control animal; c p<0.05 versus day 5 TSTQ of UA animals. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

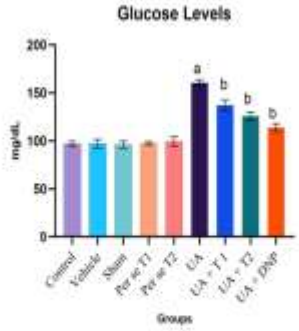

Fig 3.7: Effect of drug treatments on serum glucose levels in UA animals: Mean ± SD, n=6; Mean ± SD, n=6; one-way ANOVA followed by Tukey's test; F (8, 45) = 202.3; a p<0.001 versus glucose level of control animals, b p<0.001 versus glucose level of UA followed by treatments. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

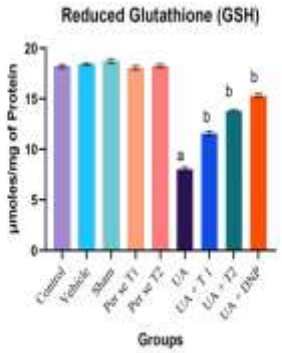

Fig 3.9: Effect of drug treatments on Brain GSH in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Tukey's multiple range test; F (8, 45) = 238.8; a p<0.05 versus control; b p<0.05 UA. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

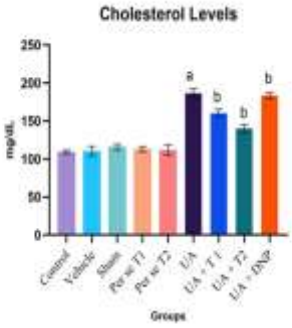

Fig 3.8: Effect of drug treatments on serum cholesterol levels in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Tukey's multiple range test; F (8,45) = 244.6; a p<0.05 versus cholesterol levels of control animals; b p<0.05 versus cholesterol levels of UA animals. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

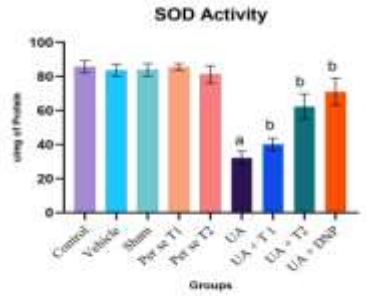

Fig 3.10: Effect of drug treatments on Brain SOD in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Tukey's multiple range test; F (8, 45) = 104.2; a p<0.05 versus control; b p<0.05 UA. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

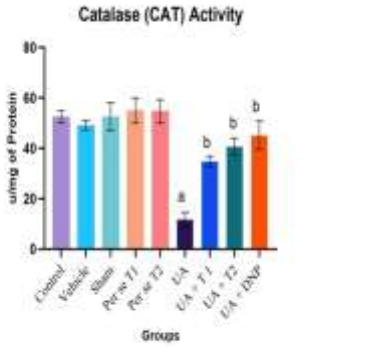

Fig 3.11: Effect of drug treatments on Brain CAT in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Tukey's multiple range test; F (8, 45) = 57.68; a p<0.05 versus control; b p<0.05 UA. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

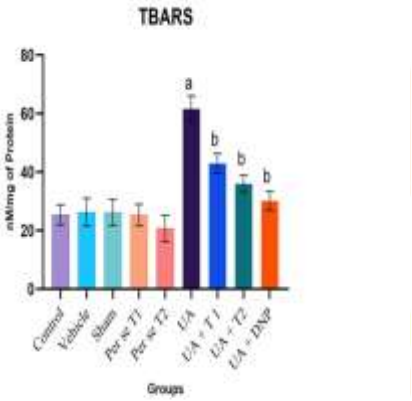

Fig 3.12: Effect of drug treatments on Brain TBARS in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Tukey's multiple range test; F (8, 45) = 64.21; a p<0.001 versus control; b p<0.001 UA. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

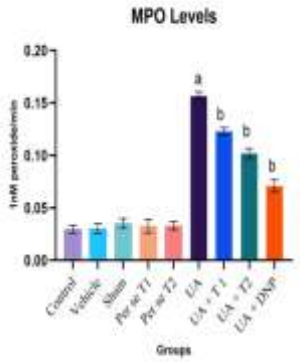

Fig 3.13: Effect of drug treatments on Brain MPO levels in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Tukey's multiple range test; F (8, 45) = 623.9; a p<0.001 versus control; b p<0.001 UA. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

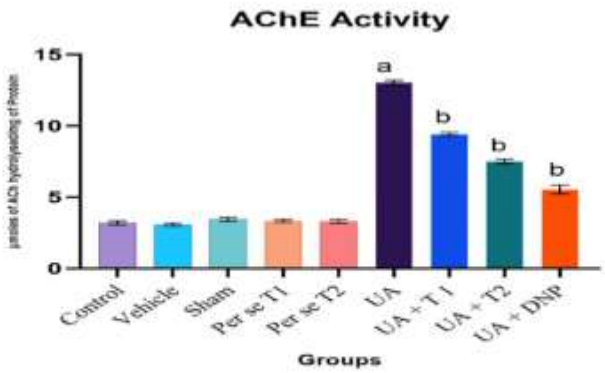

Fig 3.14: Effect of drug treatments on Brain AChE levels in UA animals: Mean ± SD, n=6; one-way ANOVA followed by Tukey's multiple range test; F (8, 45) = 280.2; a p<0.001 versus control; b p<0.001 UA. T1-Topiramate (10mg/kg); T2-Topiramate (25mg/kg); DNP-Donepezil (10mg/kg)

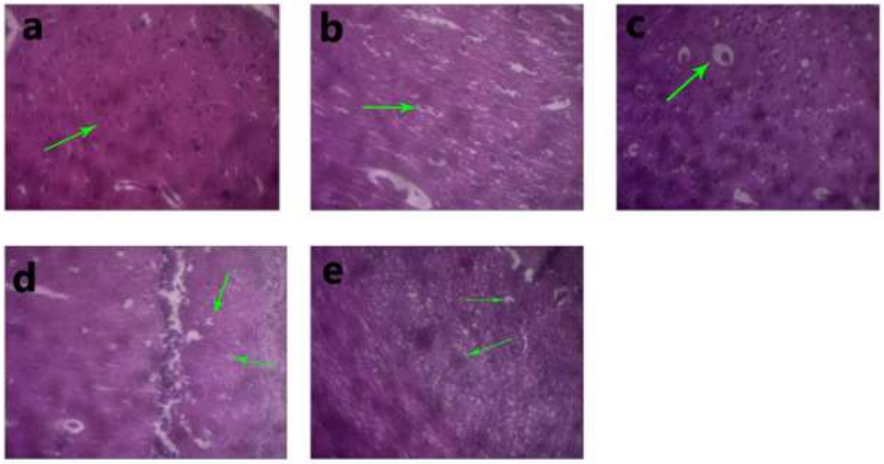

Figure 3.15: Effect of treatments on histopathological changes in the brain of UA rats. Representative photographs of H&E stained representing alterations in morphology of neurons across CAI in hippocampus area (at magnification ><400) in rat brain. **A**-sham control, **B**-Unilateral Adrenalectomized, **C**-TI treated, **D**-T2 treated, **E**- Donepezil treated Group.

DISCUSSION

This study showed that GABA receptor involvement is necessary for memory and that, at high concentrations, it may induce cytotoxicity in neural brain cells, which leads to neurodegeneration. Topiramate raises the amount of antioxidants and modifies the expression of certain receptors, which, in the case of vascular dementia, improves the endothelium layer of blood vessels in the brain, hence reducing neurodegeneration.[26,27]

In this study about 180-250g Wistar rat weighing were used in the study. The animals were split up into nine groups, each with six animals (n=6). The initial group was considered the standard control group, while the group second was the vehicle control group. The animals in the second group were treated with normal saline. The third group is for the SAM control animals they have received only incision on the body. The animals in the fourth and fifth groups were administered Topiramate separately in high and low dose respectively. [28,29]

The sixth group of rats was subjected to a unilateral adrenalectomy and allowed to recover for ten days. Adrenalectomy involves removing one adrenal gland, which might elevate stress levels. The seventh and eighth groups got Topiramate as specified in the protocol. Donepezil therapy was administered in group 9, which was considered standard treatment. After 14 days of medication therapy, behavioral evaluations were conducted utilizing the Morris water maze and elevated plus maze on protocol days. After the experimental protocol was completed, blood samples were collected to estimate glucose and cholesterol levels.[30,31]

Cervical dislocation was used to sacrifice the animals. After being extracted, the brain was homogenised for biochemical analysis, including TBARS, glutathione (GSH) activity, SOD, Catalase, AChE, and MPO. Morris Water Maze, used to measure dysregulation of memory retention. Body weight was increased more in all UA-induced animals as compared to the normal, vehicle, and per se groups. Standard drug treatment with donepezil shows moderate improvement in UA induced animals.[32,33] There is no significant difference in motor coordination and locomotor activity between induction and drug-treated animals, compared to the normal and vehicle control groups. [34,35]

The animal's performance in discovering the escape platform is dependent on the consolidation of learning memory (from hippocampus). Animals' ability to achieve rewards after pursuing a confined path relies on the consolidation of declarative memory (From cerebral cortex) and skilled memory (from basal ganglia). In the current investigation, rats with UA induction showed impairments in working and episodic memory. Because of these deficits, memories from training are completely erased.[36,37,38]

The current investigation found that UA-induced animals exhibit memory impairment on the last day of each evaluation. MWM (Morris Water Maze), however was employed to assess memory and contextual learning retention in albino wistar rats following UA-induced neurodegeneration in the form of escape latency, time spend in the target quadrant. UA induced rats had a markedly reduced capacity for memory consolidation and retention in comparison to the group under control. Undergoing Topiramate medication (low 10 mg/kg/day, p.o), and (high 25 mg/kg/day, p.o), demonstrates that rats' performance in terms of memory consolidation and retention was much improved as compared to the induction group. When rats with UA-induced memory impairment are given the conventional medication donepezil (10

mg/kg/day, oral), their memory function improves somewhat in comparison to the group that received induction treatment.[39,40,41]

The findings obtained from the Elevated Plus Maze are dependent on short-term memory. This assessment demonstrates dose-dependent improvement. As a result, animals treated with UA exhibited a substantial loss in short-term memory recall. The administration of Topiramate (low 10 mg/kg per-day, orally), and (high 25 mg/kg per-day, orally), results in a noticeably longer time spent recalling events. The time it takes to recall events rises when UA-induced animals' causes are given the standard medication donepezil (10 mg/kg/day, oral). When UA-induced induction mice were compared to normal and per se grouped animals, blood glucose and cholesterol levels increased considerably. Whereas on treatment with Topiramate (low 10 mg/kg per-day, orally), and (high 25 mg/kg per-day, orally), enhanced the levels of cholesterol and blood sugar. Standard drug treatment with donepezil (10 mg/kg/day, oral) demonstrates notable reductions in blood glucose levels but no change in total cholesterol levels.[42,43,44]

Oxidative stress increased levels are demonstrated by a progressively low brain glutathione (GSH) level. During the time of comparing the GSH levels of UA-induced rats to those of normal animals, a substantial drop was observed. On treatment with Topiramate (low 10 mg/kg per-day, orally), and (high 25 mg/kg per-day, orally), was capable of considerably increase the magnitudes of GSH in rats as compared to those brought on by UA. Standard drug therapy with donepezil (10 mg/kg/day, oral) shown a modest rise in GSH concentrations in contrast to the group UA-induced.[45,46,47,]

MPO functions as an indication of oxidative damage and inflammation increased level suggested increased ROS production. When compared to the normal group, the UA-induced animals displayed higher levels of MPO. Whereas on treatment with Topiramate (low 10 mg/kg per-day, orally), and (high 25 mg/kg per-day, orally), causes significant reduction of dose dependent manner in comparison to UA induced animals. Compared to the normal group, the donepezil-treated group shows improvement in the increased level of MPO. The vehicle, Per se and the SAM control animals did not exhibit any notable changes. [48,49,50]

CONCLUSION

This study demonstrates that GABA exerts significant neuroprotective effects in a unilateral adrenalectomy (ADX)-induced model of cognitive impairment. By restoring glutathione levels and mitigating oxidative stress, GABA not only enhances redox homeostasis but also preserves hippocampal neuronal architecture. These effects, coupled with improved behavioral outcomes, underscore the therapeutic potential of GABAergic modulation in stress-induced neurodegeneration. The findings open new avenues for exploring GABA analogs as adjunctive treatments for dementia-like conditions precipitated by corticosteroid deficiency.

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