

Research Paper

***Tetrapleura tetraptera* Extract Ameliorates Cognitive Decline and Neurodegeneration on the Pre-Frontal Cortex in an Aluminium Chloride-Induced Rat Model of Alzheimer's Disease**

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ABSTRACT

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment, neuronal degeneration, oxidative stress, and neuroinflammation.

Aim:

*To investigate the effects of *Tetrapleura tetraptera* extract (TTE) on the prefrontal cortex following aluminum chloride (AlCl₃)-induced Alzheimer's-like disease in adult male Wistar rats.*

Methodology:

Fifty (50) adult male Wistar rats were randomly assigned into five groups (n = 10). Group I (control) received 2 mL/kg body weight (bw) distilled water. Group II received 200 mg/kg bw AlCl₃, while Groups III and IV received 200 mg/kg bw AlCl₃ plus 250 and 500 mg/kg bw TTE, respectively. Group V received 10 mg/kg bw Donepezil. All treatments were administered orally for 42 days. Following treatment, rats were sacrificed, and the prefrontal cortex was harvested for histological, biochemical, and protein analyses.

Results:

TT significantly improved behavioral alterations in the elevated plus maze and Morris water maze tests (P<0.05), while reducing tissue amyloid-beta (AB), Glia Fibrillary Acidic protein (GFAP) and phosphorylated tau (p-tau) levels (P<0.05). TT significantly decreased malondialdehyde (MDA) and increased Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels (P<0.05). Neuronal and cortical restoration was observed in TT-

treated groups compared with the $AlCl_3$ group, with greater effects at the high dose. Donepezil similarly restored antioxidant enzyme activities, reduced protein deposits, and improved neuronal and cyto-architectural organization, comparable to high-dose TT.

Conclusion:

TT exhibited neuroprotective effects against $AlCl_3$ -induced Alzheimer's-like disease by mitigating oxidative stress and preserving prefrontal cortical integrity.

KEYWORDS: *Tetrapleura tetraptera*; Oxidative stress; Prefrontal cortex; Alzheimer's Disease; Neurodegeneration.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and a progressive neurodegenerative disorder characterized by a gradual decline in memory, cognition, learning ability, and executive function¹. It accounts for approximately 60–70% of all dementia cases worldwide and constitutes a major public health challenge due to increasing life expectancy and population aging². The pathological hallmarks of Alzheimer's disease include extracellular deposition of amyloid- β ($A\beta$) plaques, intracellular accumulation of hyper phosphorylated tau protein in the form of neurofibrillary tangles, synaptic dysfunction, neuronal loss, neuroinflammation, oxidative stress, and progressive cerebral atrophy³.

Oxidative stress is recognized as one of the central mechanisms underlying aluminum-induced neurodegeneration⁴. Excessive generation of free radicals overwhelms endogenous antioxidant defense systems, resulting in lipid peroxidation, protein oxidation, DNA damage, and ultimately neuronal death^{5,6}. In addition to oxidative injury, aluminum exposure activates inflammatory pathways involving microglial activation and astrocytes proliferation, leading to increased production of pro-inflammatory cytokines that exacerbate neuronal damage⁶. Consequently, therapeutic strategies possessing antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective properties are increasingly being explored as potential interventions against Alzheimer's disease⁷.

Tetrapleura tetraptera, commonly known as Aidan fruit, is a medicinal plant indigenous to West and Central Africa and has been widely utilized in traditional medicine for the management of numerous ailments⁸. The fruit contains a rich array of bioactive phytochemicals, including phenolic compounds, flavonoids, tannins, alkaloids, saponins, and essential oils⁹. Several experimental studies have demonstrated that extracts of *Tetrapleura tetraptera* effectively scavenge reactive oxygen species, enhance endogenous antioxidant enzyme activities, suppress inflammatory mediators, and reduce oxidative tissue damage in various experimental models¹⁰⁻¹².

Despite the growing body of literature describing the pharmacological properties of *Tetrapleura tetraptera*, limited information exists regarding its protective effects on the prefrontal cortex following aluminum chloride-induced Alzheimer's-like neurodegeneration. Most previous investigations have focused primarily on general antioxidant activity or hippocampal protection, while relatively few studies have comprehensively evaluated the structural preservation of the prefrontal cortex. Given the crucial role of the prefrontal cortex in cognition and executive function, investigating the effects of *Tetrapleura tetraptera* on this brain region is of considerable scientific and therapeutic importance. Therefore, this study aims to evaluate the effects of *Tetrapleura tetraptera* on the prefrontal cortex following aluminum chloride-induced Alzheimer's-like disease in adult male Wistar rats.

MATERIALS AND METHODS

Materials

Experimental Chemicals

Aluminum Chloride ($AlCl_3$): 20 g $AlCl_3$ produced by Span Chemie, India and was gotten from Dennis and Dennis Store Ilorin, Kwara State and authenticated in the Department of Chemistry, Faculty of Natural and Applied Sciences, Al-Hikmah University, Ilorin.

Donepezil: 10 gm Donepezil: was bought from One-Step Pharmacy Ilorin, Kwara State.

Experimental Plant: 1000 g of *Tetrapleura tetraptera* was obtained from Oja Oba market Osogbo, Osun State where it was powdered and the seed were removed and was taken to the Department of Industrial Chemistry, Faculty of Physical Science, University of Ilorin, and Kwara State for extraction.

Experimental Animals: Fifty (50) apparently healthy Wistar rats were purchased from the animal facility of Department of Human Anatomy, Faculty of Basic Medical Science, College of Health Science, Osun State University, Osogbo, Osun State, Nigeria. To replicate their natural habitat, the Wistar rats were housed at ambient temperature with a typical light-dark cycle (12:12). To suit their nutritional needs, the Wistar rats were

given purified water and grower pellet diet during the trial. Two weeks prior to the start of the experiment, the Wistar rats were acclimated.

Other Materials: Syringes, gloves, sample bottles, distilled water and dissecting kits were of analytical grades.

METHODS

Ethical Approval

All experimental procedures were carried out in accordance with the regulation stipulated by the Health Research Ethics Committee of College of Health Sciences, Osun State University Osogbo, Osun State, Nigeria, in conformity with the Guidelines of the National Institutes of Health for the care and use of laboratory Animals.

Experimental Protocol

Fifty (50) adult male Wistar rats were randomly assigned into five groups ($n = 10$). Group I (control) received 2 mL/kg body weight (bw) distilled water. Group II received 200 mg/kg bw $AlCl_3$, while Groups III and IV received 200 mg/kg bw $AlCl_3$ plus 250 and 500 mg/kg bw TTE, respectively. Group V received 10 mg/kg bw Donepezil. All treatments were administered orally for 42 days. The rats were kept under standard condition of 12 hours dark and day cycle and were fed with rat feed and water *Ad libitum*. All administrations were done orally. Neurobehavioral tests (Morris Water Maze and Standard elevated plus maze) were carried out a day before sacrifice. After the last day of administration, the rats were fasted overnight and their final body weights were taken. The rats were humanely euthanized through cervical dislocation and the brains were harvested through mid-sagittal incision. The rats' brains were divided into two. Five rats' brains were homogenized in (5x W/V) of sucrose solution for biochemical and protein analysis and the others fixed in 10% formalin for histological and histochemical assays.

NEUROBEHAVIOURAL TEST

Morris Water Maze

The Morris water test (spatial memory and learning) was used to assess spatial learning and memory in the rats. The test was conducted in a circular pool (diameter 150 cm) filled with opaque water, with a hidden platform submerged just below the surface. Each rat was given three trials per day for 12 days to locate the platform. The time taken each rat to find the platform (escape latency) was recorded as a measure of spatial learning ability¹³.

Standard Elevated plus Maze

The maze consisted of four arms arranged in a 'plus' shape, with two enclosed arms and two open arms, elevated 50 cm above

the ground. The open arms are 45 cm $\times$ 10 cm $\times$ 30 cm length and width respectively. The two closed arms are 45 cm $\times$ 10 cm $\times$ 30 cm, long, width and height respectively. The central platform is 10 cm $\times$ 10 cm. The rats were placed in the center of the maze, and their behavior (grooming and rearing behavior) was observed for 5 minutes. The primary outcome measure was the percentage of time spent in the open arms, with more time spent in the open arms indicating lower anxiety levels¹⁴.

Oxidative Stress Markers

Malondialdehyde (MDA)

MDA was determined using the thiobarbituric acid reactive substances (TBARS) method originally described by Ohkawa *et al*¹⁵. 0.2 mL of the supernatant was mixed with 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid (pH 3.5), and 1.5 mL of 0.8% thiobarbituric acid (TBA). The reaction mixture was heated in a boiling water bath (95–100°C) for 60 minutes and then cooled to room temperature. Distilled water (1.0 mL) and 5.0 mL of n-butanol:pyridine (15:1, v/v) were added, followed by vigorous mixing and centrifugation at 4,000 rpm for 10 minutes. The organic layer was carefully collected, and its absorbance was measured spectrophotometrically at **532 nm** against a reagent blank. MDA concentration was calculated using a standard curve prepared from 1,1,3,3-tetraethoxypropane¹⁶.

Superoxide dismutase (SOD)

SOD activity was determined according to the method of Kakkar *et al*¹⁷. 0.1 mL of tissue homogenate was added to a reaction mixture containing sodium pyrophosphate buffer (0.052 M, pH 8.3), phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), and nicotinamide adenine dinucleotide (NADH). The reaction was initiated by the addition of NADH and allowed to proceed for 90 seconds at room temperature before termination with glacial acetic acid. The chromogen was extracted using n-butanol, and absorbance was measured at **560 nm** using a UV-visible spectrophotometer

Catalase (CAT)

Catalase activity was determined using the method described by Aebi¹⁸. Brain tissue homogenates were prepared in ice-cold phosphate buffer (50 mM, pH 7.0) and centrifuged at $10,000 \times g$ for 15 minutes at 4°C. The reaction mixture consisted of phosphate buffer, freshly prepared hydrogen peroxide (30 mM), and tissue supernatant. Catalase activity was determined by monitoring the decomposition of hydrogen peroxide spectrophotometrically at **240 nm** for 3 minutes. The decrease in absorbance per minute was recorded, and enzyme activity was calculated using the molar extinction coefficient of hydrogen peroxide ($43.6 M^{-1} cm$).

Glutathione peroxidase (GPx)

GPx activity was estimated using the method of Rotruck et al.¹⁹ Briefly, tissue homogenate was incubated with phosphate buffer (0.4 M, pH 7.0), sodium azide, reduced glutathione (GSH), hydrogen peroxide, and ethylenediaminetetraacetic acid (EDTA). The reaction mixture was incubated at 37°C for 10 minutes to allow enzymatic reduction of hydrogen peroxide by GPx. The reaction was terminated with trichloroacetic acid (TCA), followed by centrifugation. The remaining reduced glutathione in the supernatant was reacted with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), producing a yellow-colored complex. Absorbance was measured at **412 nm** using a spectrophotometer.

Apoptotic and Neurodegenerative Markers

Determination of Bcl-2

The concentration of B-cell lymphoma-2 (Bcl-2) was carried out using ELISA kit. 100 µL tissue were added into microplate wells pre-coated with monoclonal antibodies specific for rat Bcl-2 and incubated at 37°C for 90 minutes. Following washing to remove unbound proteins, biotin-conjugated anti-Bcl-2 detection antibody was added and incubated for 60 minutes. After additional washing, horseradish peroxidase (HRP)-streptavidin conjugate was added and incubated for 30 minutes. Tetramethylbenzidine (TMB) substrate solution was subsequently added and allowed to react in the dark for 15–20 minutes before the reaction was terminated using stop solution. Optical density was measured at 450 nm using a microplate reader. Bcl-2 concentrations were calculated from a standard calibration curve²⁰.

Determination of Bax

Bax protein concentration was determined using a rat-specific sandwich ELISA kit according to the manufacturer's protocol. Brain tissues were homogenized in ice-cold PBS containing protease inhibitors and centrifuged at 10,000 × g for 15 minutes at 4°C. The resulting supernatant was collected for assay. 100 µL of tissue homogenates were pipetted into antibody-coated wells and incubated for 90 minutes at 37°C. Following washing, biotin-labelled anti-Bax antibody was added, followed by HRP-conjugated streptavidin. The antigen-antibody complex was visualized using TMB substrate and the enzymatic reaction was terminated with acidic stop solution. Absorbance was read at 450 nm, and Bax concentrations were calculated from the standard curve^{21,22}.

Determination of Caspase-3

Caspase-3 levels were quantified using ELISA kit. One hundred microliters added to antibody-coated wells and incubated at 37°C for 90 minutes. Wells were washed thoroughly before incubation with biotinylated anti-caspase-3 antibody, followed by HRP-labelled streptavidin. After washing, TMB substrate solution was added, and colour development was allowed to proceed for approximately 15 minutes in the dark. The reaction was terminated using stop solution, and absorbance was measured at 450 nm using a microplate reader. Caspase-3 concentrations were obtained from the standard curve^{23,24}.

Determination of Glial Fibrillary Acidic Protein (GFAP)

GFAP concentration was determined using a ELISA kit. Tissue homogenates were transferred into microplate wells coated with anti-GFAP capture antibody and incubated for 90 minutes at 37°C. Following washing, biotin-labelled detection antibody and HRP-streptavidin conjugate were sequentially added with incubation and washing steps between each stage. Colour was developed using TMB substrate and stopped with acidic stop solution. Optical density was measured at 450 nm using a microplate spectrophotometer. GFAP concentrations were determined from the standard curve²⁵.

Determination of Phosphorylated Tau (p-Tau)

Phosphorylated tau protein (p-Tau) was quantified using ELISA kit. Tissue homogenates were added into antibody-coated wells and incubated at 37°C for 90 minutes. Following washing, biotinylated anti-p-Tau detection antibody was added, followed by HRP-streptavidin conjugate. TMB substrate solution was added for colour development, and the reaction was stopped using sulfuric acid stop solution. Absorbance was measured at 450 nm. Protein concentrations were calculated using a standard curve²⁶.

Determination of Amyloid-β

Amyloid-β (Aβ), quantified using ELISA kit 100 µL were added to wells coated with anti-Aβ capture antibody and incubated for 90 minutes at 37°C. Following washing, biotinylated anti-Aβ detection antibody and HRP-conjugated streptavidin were added sequentially. Colour development was achieved using TMB substrate, and the reaction was terminated with stop solution. Absorbance was measured at 450 nm, and Aβ concentrations were calculated from a standard curve²⁷.

RESULTS

Physical Observation

Induction Phase

The rats in the control group remained healthy and active throughout the experimental period. Normal grooming, rearing, feeding behavior, and locomotor activities. No visible signs of toxicity, inflammation, paralysis, or mortality were recorded throughout the study period.

However, the rats in the $AlCl_3$ treated groups exhibited behavioral and neurological abnormalities including reduced activity, calmness, weakness, and excessive grooming. By Day 24, one rat became temporarily unconscious and later developed difficulty in movement.

Between Days 24 and 28, some rats showed obvious neurological signs such as sideways walking, imbalance, rapid breathing (tachycardia), and partial paralysis of the limbs. Progressive enlargement of the scrotum was observed in several rats across the groups, while swelling of the nose, face, and tail became more pronounced in this week.

Treatment Phase

From Days 29 to 42, the rats in $AlCl_3$ only Group revealed worsened inflammatory and or toxicity signs including swollen nose, face, tail, scrotal bleeding and scleritis. One rat also developed a cut on its tail. Body weight reduction and poor feeding behavior became noticeable during this period. More so, loss of normal movement, paralysis, reduced feed intake, scrotal swelling, weakness, and decreased locomotor activity.

Following administration of low-dose TT, gradual improvement in feeding pattern, grooming behavior, and

movement was observed. Some animals regained normal rearing activity and became more active toward the later stage of treatment, although mild weakness and swelling persisted in a few rats.

More so, upon treatment with high-dose TT group, improvement in locomotor activities, grooming behaviors, and feeding response became more evident with recovery from neurological symptoms and inflammatory changes appeared faster and more pronounced in this group when compared to the low-dose TT treated group. Meanwhile, Donepezil administration, improved movement, grooming, and feeding behavior. Although mild inflammatory signs such as scrotal swelling persisted in some rats toward the end of the experimental period.

Organ and Body Weight

There were no significant differences in initial body weight among experimental groups ($P = 0.07$), confirming homogeneity before treatment. However, following the experimental period, final body weight differed significantly ($P < 0.05$). Rats exposed to $AlCl_3$ exhibited the smallest weight gain, suggesting impaired growth and systemic toxicity. In contrast, both TT-treated groups demonstrated greater weight gain, particularly the high-dose group, indicating improved physiological status after treatment.

Brain weight (organ weight) did not differ significantly among groups. Although TT-treated rats tended to have slightly greater brain weights than the $AlCl_3$ group. This suggests that while $AlCl_3$ primarily affected brain function rather than gross morphology, TT may have exerted a mild protective effect against tissue loss [Table 1].

Table 1: Initial, Final Body and Brain Weights of Rats across the Groups

GROUPS & TREATMENT	INITIAL BW (g)	FINAL BW (g)	BRAIN WEIGHT (g)
A (Control)	213.857 ± 5.353	239.286 ± 4.934	1.53 ± 0.12
B ($AlCl_3$)	212.286 ± 6.149	218.286 ± 5.017*	1.554 ± 0.23
C ($AlCl_3$ + Low Dose TT)	210.857 ± 5.075	229.571 ± 4.256	1.768 ± 0.14
D ($AlCl_3$ + High Dose TT)	215.429 ± 5.103	235.429 ± 15.784	1.654 ± 0.34
E ($AlCl_3$ + Donepezil)	213.143 ± 6.092	227.143 ± 4.647	1.7945 ± 0.13

Data expressed as Mean ± SEM. BW (Body Weight)

Asterisk (*) indicates statistically significant differences.

Morris Water Maze Test (Spatial Learning and Memory)

The findings demonstrated no statistically significant differences among the experimental groups during the training phase ($P = 0.077$), indicating that all rats possessed comparable learning capacity before disease induction. However, following aluminum chloride (AlCl_3) administration, escape latency during the induction phase increased markedly from 8.37 ± 1.74 s in the control group to 18.43 ± 0.78 s in the AlCl_3 -treated group ($P = 0.037$), demonstrating significant impairment of spatial learning. Meanwhile, rats that received AlCl_3 alone exhibited significant increase in escape latency (28.57 ± 4.36 s), indicating persistent cognitive dysfunction. Conversely, treatment with TT significantly reduced escape latency to 8.00 ± 0.44 s in the low-dose group and 7.00 ± 0.31 s in the high-dose group. Similarly, donepezil-treated animals showed improved performance (8.14 ± 0.91 s). These improvements were statistically significant ($P = 0.005$) [Table 2].

Standard Elevated Plus Maze Test

The results revealed significant alterations in anxiety-related behavior following AlCl_3 administration. Rats treated with AlCl_3 spent only 2.86% of the testing period in the open arms compared with 66.57% in control animals ($P = 0.05$), while occupancy of the closed arms increased dramatically to 97.14%. These findings indicate severe anxiety-like behavior induced by aluminum toxicity. Treatment with TT significantly increased open-arm exploration to 41.43% (low dose) and 45.71% (high dose), while reducing time spent in the closed arms. Donepezil produced similar anxiolytic effects. Although Head-dipping behavior was not significantly altered among groups, rearing activity differed significantly ($P < 0.05$), with the high-dosed group exhibiting the highest frequency, indicating restoration of exploratory locomotor behavior. More so, Aluminum-treated rats exhibited excessive grooming, a behavioral indicator of stress and anxiety, whereas TT treatment reduced grooming frequency toward normal control values [Table 3].

Table 2: T, IPEL and TPEL in Morris Water Maze Test

S.No.	GROUPS & TREATMENT	T (sec)	IPEL (Sec)	TPEL (Sec)
1.	A (Control)	7.714 ± 0.474	$8.37 \pm 1.74^*$	5.857 ± 0.340
2.	B (AlCl_3)	7.429 ± 0.481	18.429 ± 0.781	$28.571 \pm 4.358^*$
3.	C (AlCl_3 + Low Dose TTE)	7.286 ± 0.421	15.286 ± 0.121	8.000 ± 0.436
4.	D (AlCl_3 + High Dose TTE)	9.419 ± 0.520	12.419 ± 0.320	7.000 ± 0.309
5.	E (AlCl_3 + Donepezil)	6.714 ± 0.993	16.714 ± 0.295	8.143 ± 0.911

Data expressed as Mean $\pm$ SEM

T (Training), IPEL (Induction Escape Latency), TPEL (Treatment Phase Escape Latency)

Asterisk (*) indicates statistically significant differences.

Table 3: PPOA, PPCA, HD, RF and GF in Standard Elevated Plus Maze Test

GROUPS & TREATMENT	PPOA(%)	PPCA(%)	HD	RF	GF
A (Control)	66.571 ± 4.041	33.429 ± 4.041	2.143 ± 0.911	5.286 ± 1.017	5.430 ± 1.020
B (AlCl_3)	$2.857 \pm 2.857^*$	$97.143 \pm 2.857^*$	1.429 ± 1.152	$3.000 \pm 0.976^*$	$12.000 \pm 0.816^*$
C (AlCl_3 + Low Dose TT)	41.429 ± 4.041	58.571 ± 4.041	4.286 ± 0.565	6.000 ± 0.976	3.857 ± 0.340
D (AlCl_3 + High Dose TT)	45.714 ± 8.411	54.286 ± 8.411	3.429 ± 0.369	$8.143 \pm 0.634^*$	5.571 ± 0.481
E (AlCl_3 + Donepezil)	43.0 ± 10.690	56 ± 10.690	3.571 ± 0.751	7.857 ± 0.884	3.429 ± 0.649

Data expressed as Mean $\pm$ SEM

PPOA (Percentage permanency in the open arm), PPCA (Percentage Permanency in the Close Arm), HD (Head Dip),

RF (Rearing Frequency), GF (Grooming Frequency)

Asterisk (*) indicates statistically significant differences.

BIOCHEMICAL STUDIES

Oxidative Stress Markers

The AlCl_3 -treated group showed a significant increase in malondialdehyde (MDA) compared with the control ($p < 0.05$), indicating enhanced lipid peroxidation and oxidative stress. Conversely, the activities of the endogenous antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were significantly decreased ($p < 0.05$), demonstrating impairment of the antioxidant defense system. However, treatment with low-dose TT significantly reduced MDA while significantly increasing SOD, CAT, and GPx activities compared with the AlCl_3 group ($p < 0.05$). Although antioxidant activities improved, they remained below control values, suggesting partial restoration of oxidative balance. Administration of high-dose TT produced greater reductions in MDA and more pronounced increases in SOD, CAT, and GPx activities than the low-dose treatment ($p < 0.05$), indicating a dose-dependent antioxidant effect. The Donepezil-treated group also demonstrated significant antioxidant effects comparable to those of high-dose TT group [Figure 1].

Apoptotic/Neurodegenerative Markers

The AlCl_3 -treated group exhibited a significant increase ($p < 0.05$) in amyloid- β ($\text{A}\beta$), phosphorylated tau (pTau), GFAP, Bax, and Caspase-3 compared with the control group, while Bcl-2 expression was significantly reduced. These findings indicate that aluminum chloride successfully induced Alzheimer's-like neuropathology characterized by amyloid deposition, tau hyper phosphorylation, astrocyte activation, and increased neuronal apoptosis.

Furthermore, administration of low-dose TT significantly reduced $\text{A}\beta$, pTau, GFAP, Bax, and Caspase-3 levels compared with the AlCl_3 group ($p < 0.05$). However, these values remained significantly different from those of the control group, suggesting partial neuroprotection.

Similarly, the high-dose TT group demonstrated significant reductions in the neurodegenerative and apoptotic markers relative to the AlCl_3 -treated animals ($p < 0.05$). The reductions were generally greater than those observed with the low dose, indicating a dose-dependent protective effect. Bcl-2 levels were significantly increased in both TT-treated groups compared with the AlCl_3 group ($p < 0.05$), suggesting enhanced neuronal survival and inhibition of apoptosis [Figure 2].

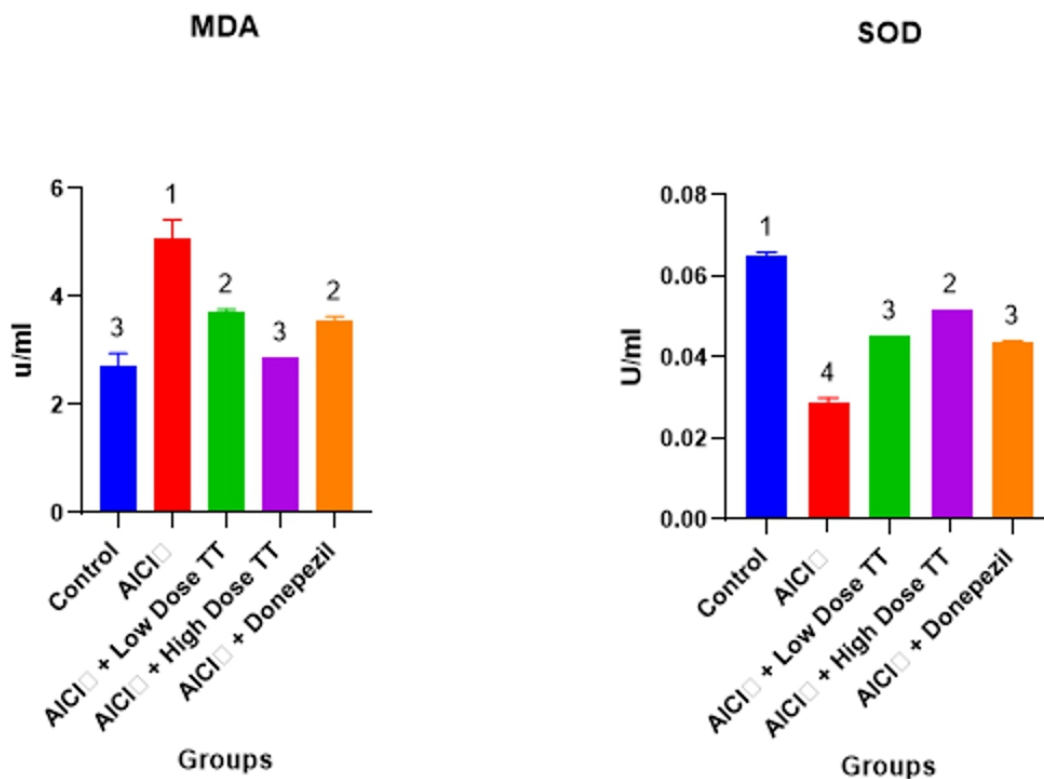


Figure 1: Bar charts showing Mean $\pm$ Standard Error of Mean (SEM) of MDA, SOD, CAT and Gpx in Experimental Rats across the Groups. (Groups bearing different superscript differ at $P < 0.05$)

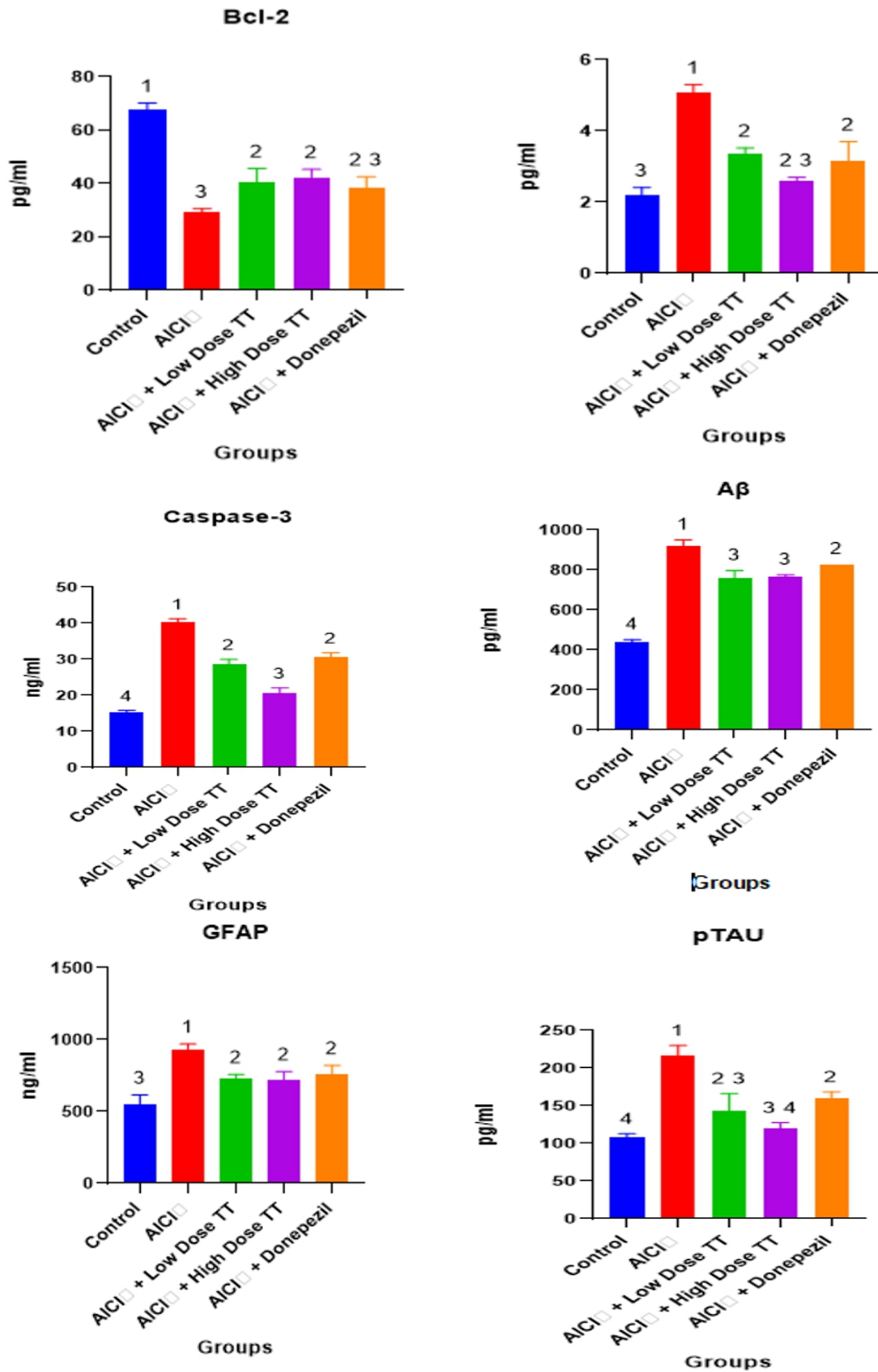


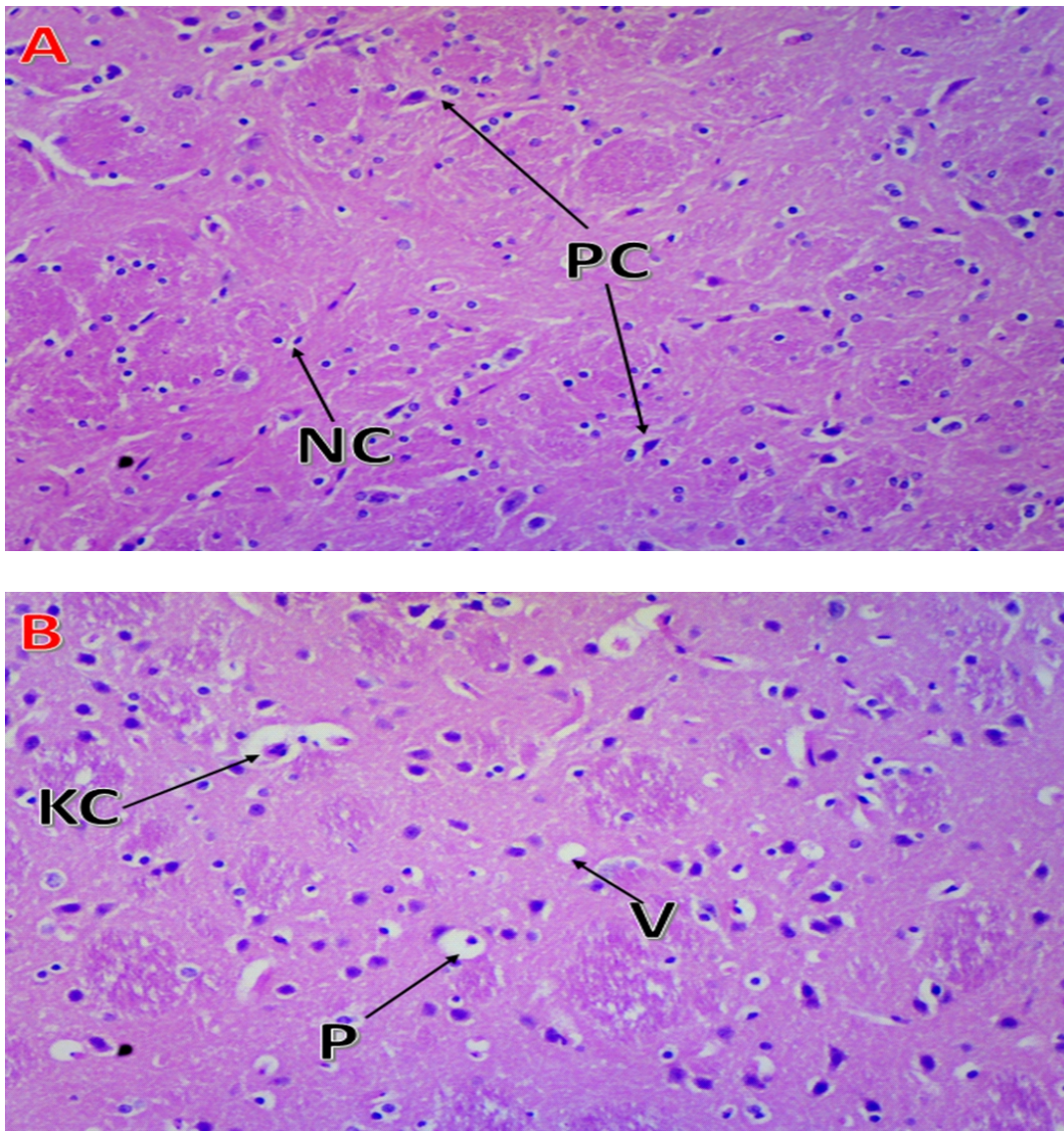
Figure 2: Bar charts showing Mean $\pm$ Standard Error of Mean (SEM) of B cell Lymphoma 2 (Bcl-2), B cell Associated X Protein (Bax), and Caspase-3, A β , GFAP and pTAU in Experimental Rats across the Groups. (Groups bearing different superscript differ at $P < 0.05$)

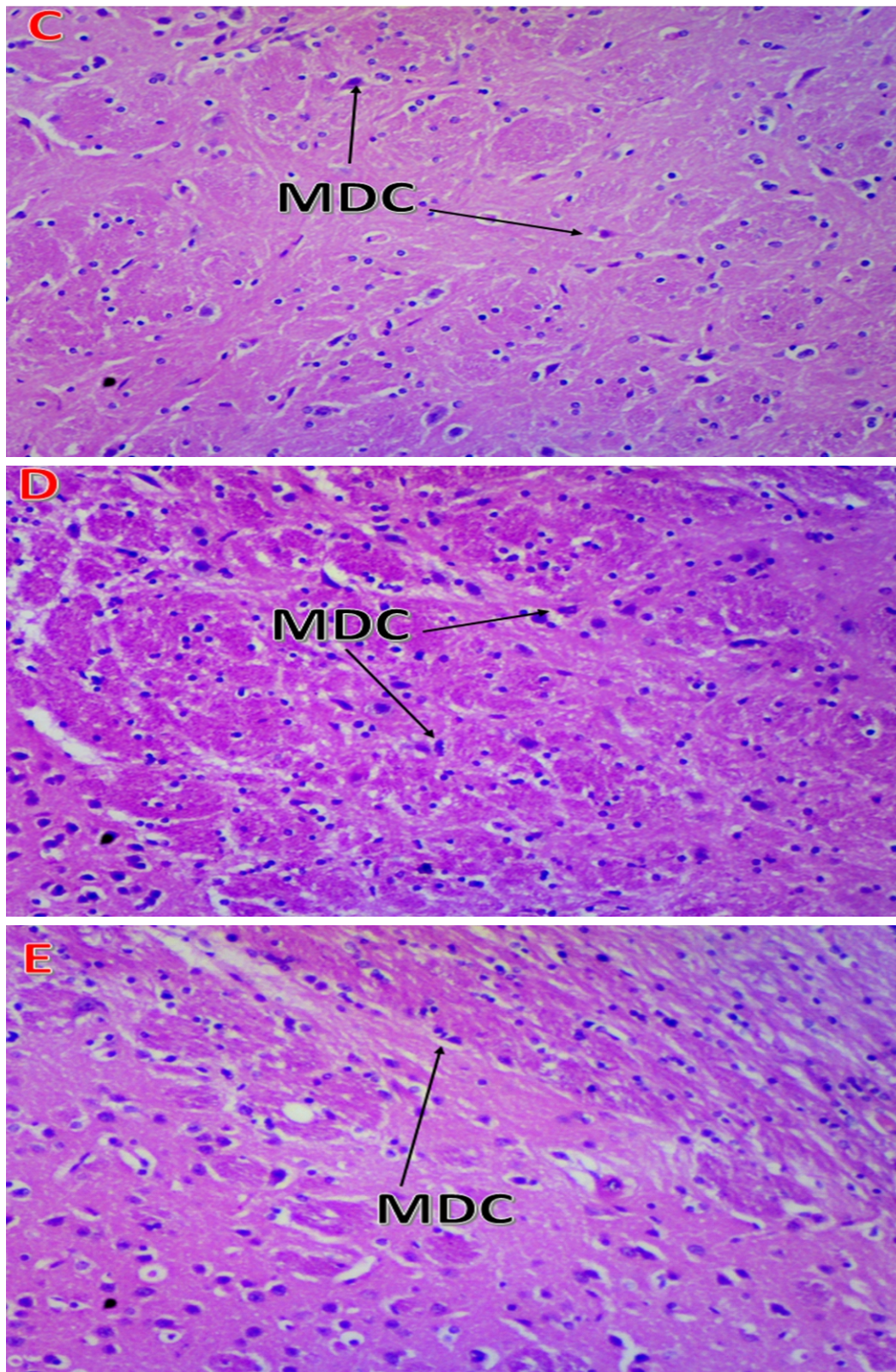
HISTOLOGICAL FINDINGS

Aluminum chloride produced profound neurodegenerative changes in the prefrontal cortex, characterized by neuronal loss, nuclear degeneration, and cytoplasmic vacuolation, impaired Nissl substance integrity (Plates B & F) and Alzheimer's disease-like pathological lesions including senile plaques (Plate L) TT substantially ameliorated these alterations, preserving neuronal architecture, reducing

neuronal degeneration (Plates C & D), maintaining Nissl substance integrity (Plates H & I) and decreasing the extent of amyloid plaque deposition (Plates M & N). The histological improvements observed in TT-treated groups were comparable to those produced by donepezil, suggesting that the TT extract possesses significant neuroprotective properties, likely mediated through its antioxidant, anti-inflammatory, and anti-apoptotic activities.

HEMATOXYLIN AND EOSIN (H&E) STAIN RESULTS





Plates A - E:

A, Showing Photomicrograph of rats in the Control group showing normal cytoarchitecture of the layer V of the prefrontal cortex with normal Pyramidal cells (PC) and normal Neuronal cells (NC). **B**, $AlCl_3$ only group showing several degrees of neurodegenerations: Karyorrhectic Cells (KC), Vacuolations (V) and Pyknosis (P). **C & D**: $AlCl_3$ + Low and High Dose TT group showing Moderate and Mild degenerations of Cells (MDC) respectively. **E**, $AlCl_3$ + Donepezil group showing areas Mild degenerations of Cells (MDC) (H&E X 100).

CRISYLE FAST VIOLET STAIN RESULTS

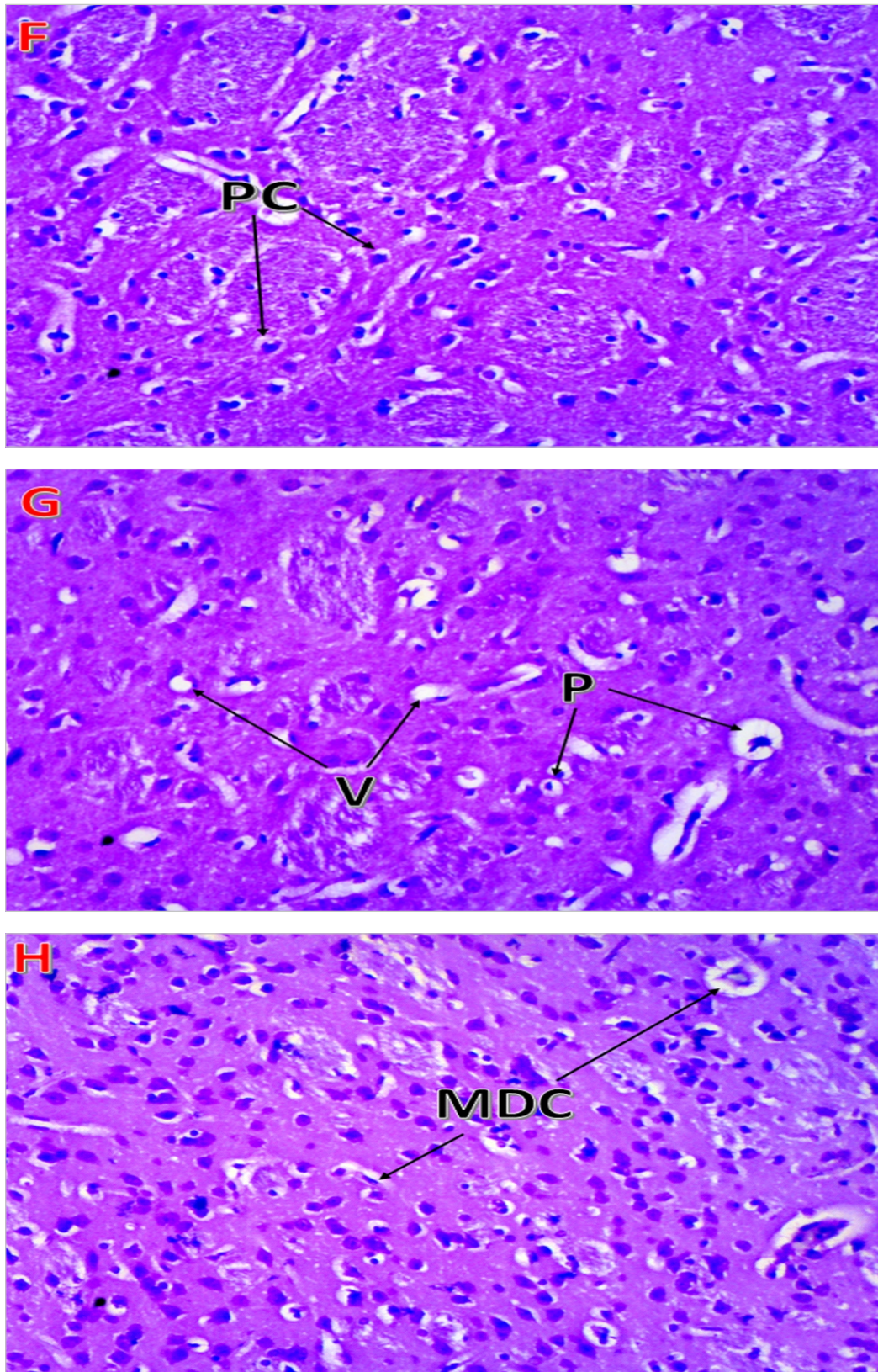
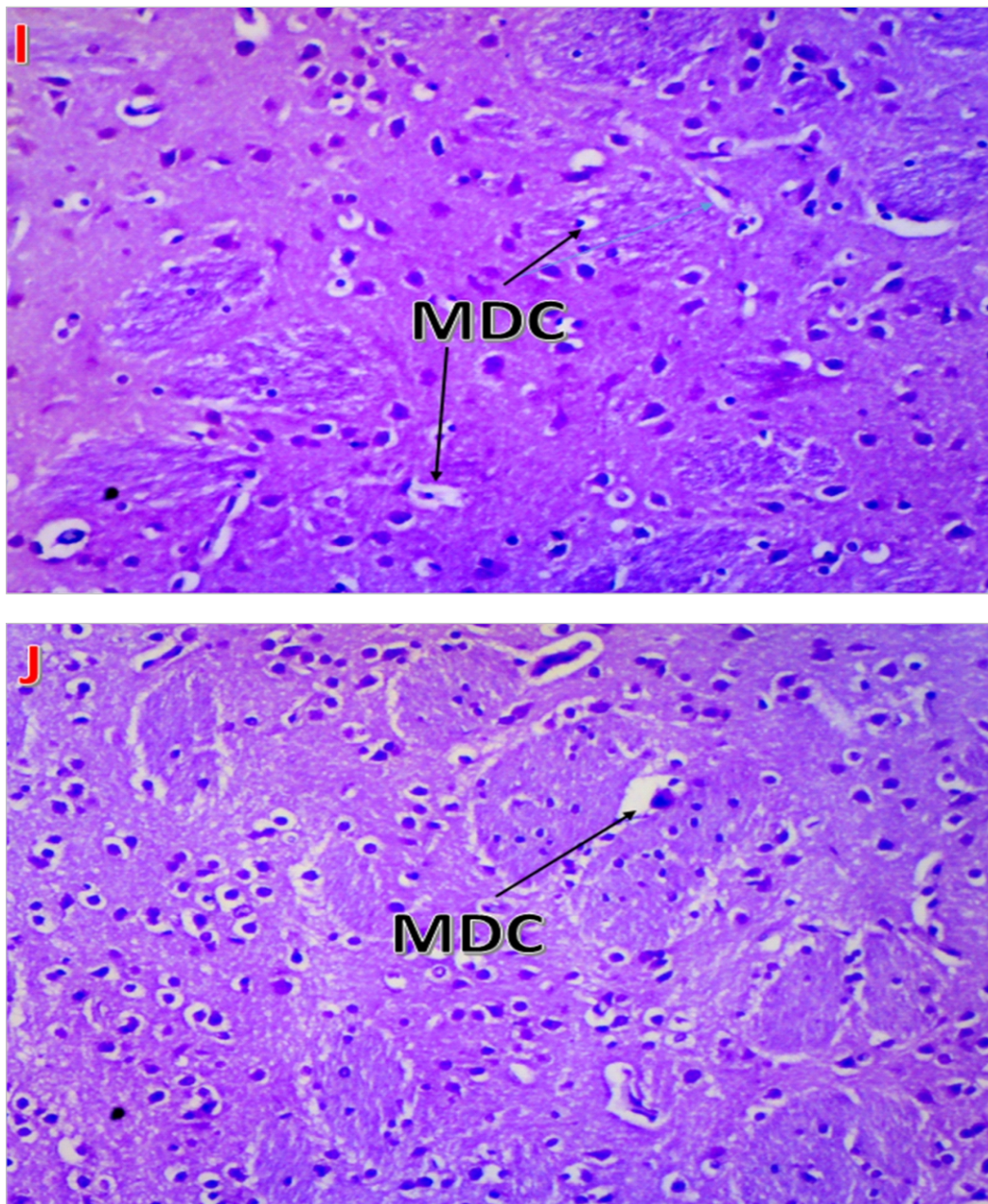


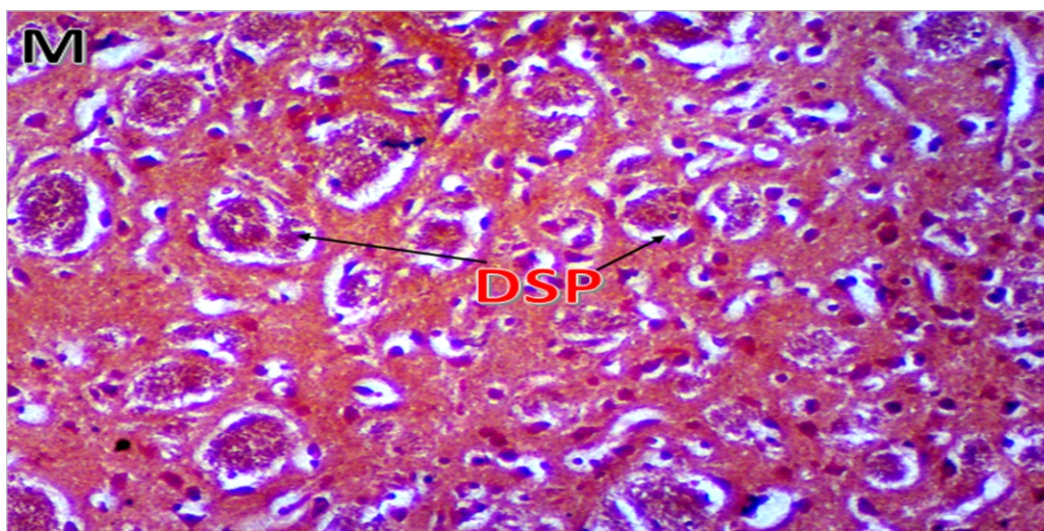
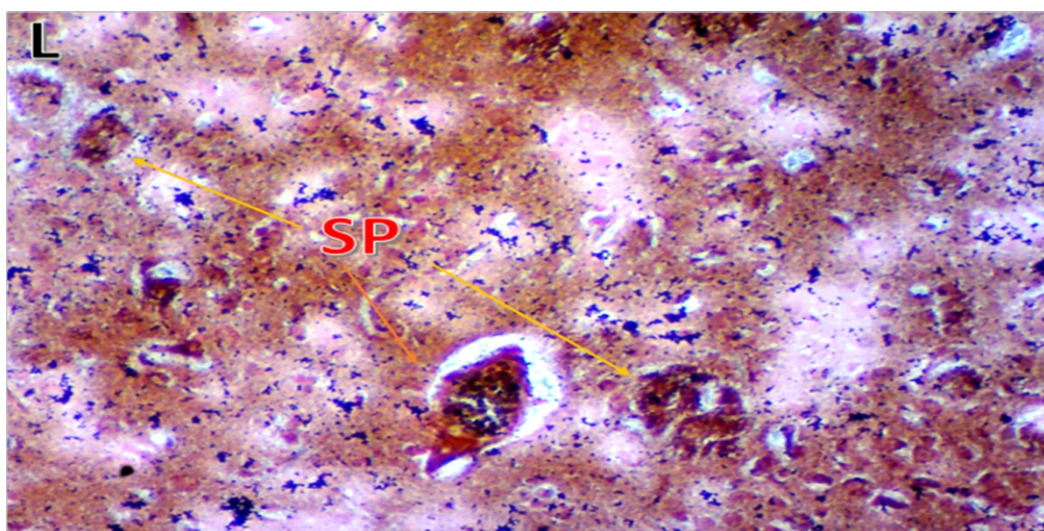
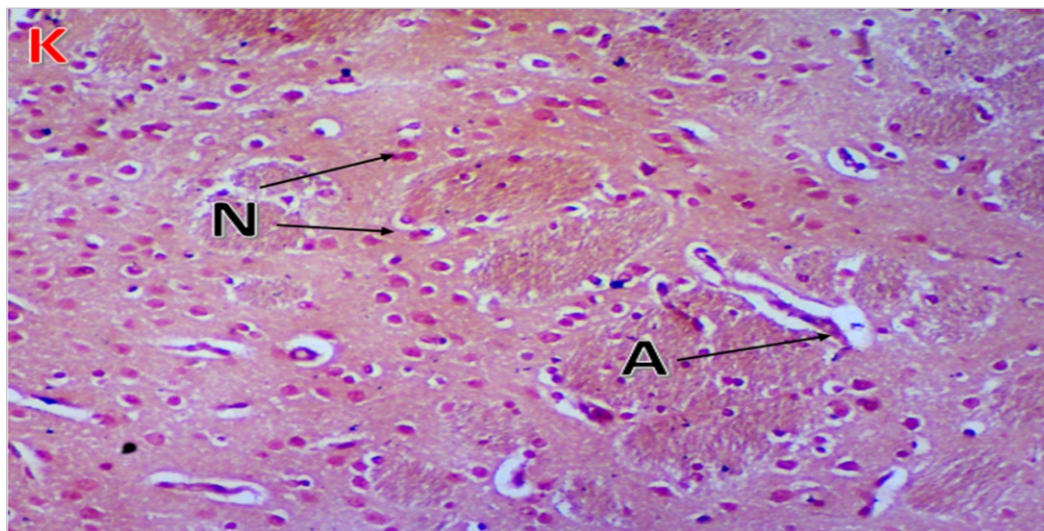
Figure 5: Algorithm for Fluid Management in Severe Dengue
Flowchart for Stepwise IV Fluid Therapy in Shock/ Plasma Leakage with Reassessment Points

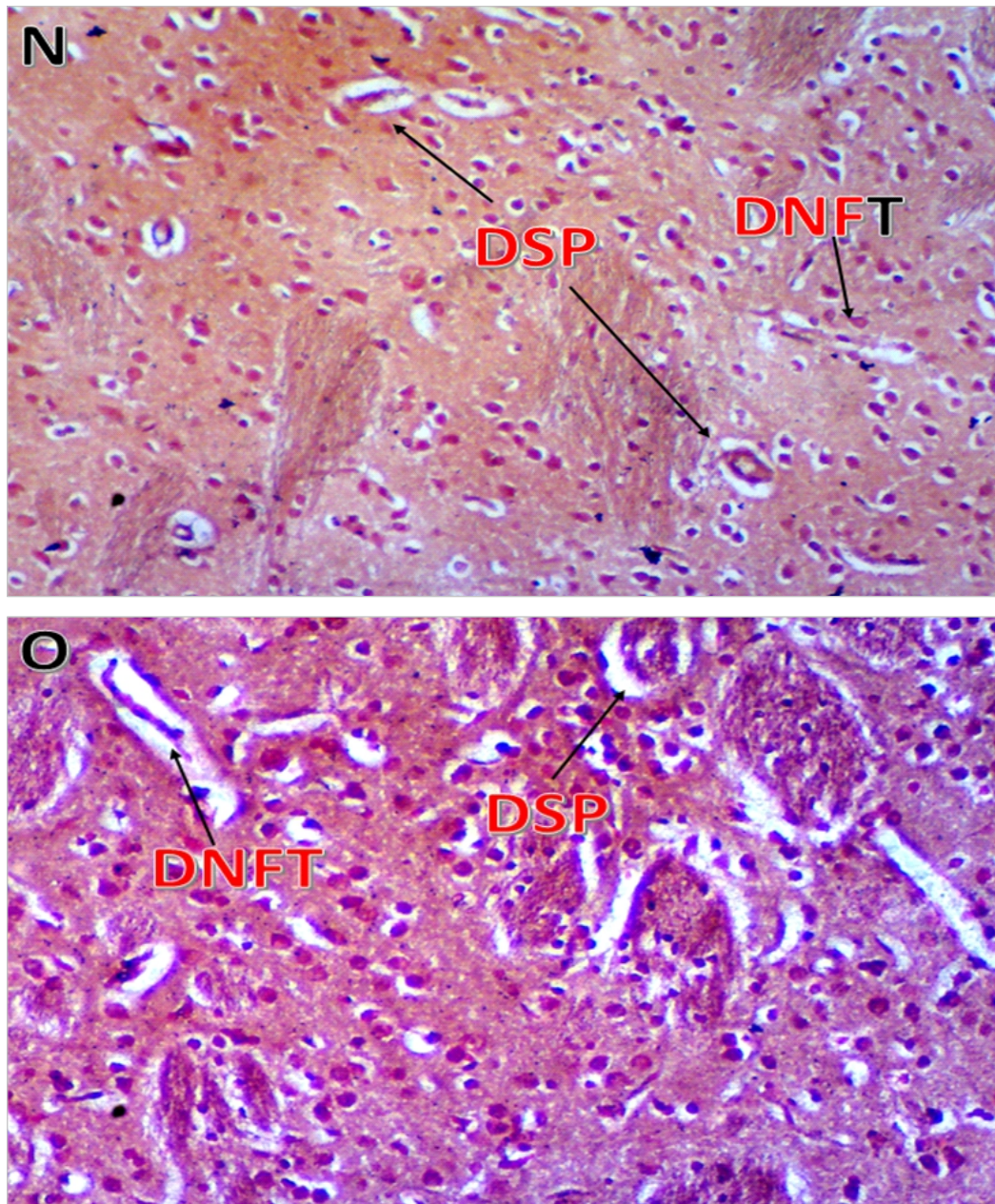


Plates F - J:

- F**, Photomicrograph of rats in the Control group showing normal cytoarchitecture of the layer V of the prefrontal cortex showing densely stained normal Pyramidal cells (PC) and normal Neuronal cells (NC).
- G**, AlCl₃ only group showing several degrees of neurodegenerations: Vacuolations (V) and Pyknosis (P),
- H & I**: AlCl₃ + Low and High Dose TT groups showing areas moderate degenerations of Cells (MDC).
- J**, AlCl₃ + Donepezil group showing areas Mild degenerations of Cells (MDC) (CFV X 100).

BIELSCHOWSKY STAIN RESULTS





Plates K - O:

K, Photomicrograph of layer V of prefrontal cortex of rats in the Control group showing brown stained normal Neurons (N) and normal cell Axons with no Senile Plaques nor Neurofibrillary Tangles (A). **L**, AlCl₃ only group showing deep brown-stained Senile Plaques (SP). **M & N**, AlCl₃ + Low & High Dose TT groups showing moderate Diffusing Senile Plaques (DSP) and Neurofibrillary Tangles (DNFT). **O**, AlCl₃ + Donepezil group showing mild Diffusing Senile Plaques (DSP) and Neurofibrillary Tangles (DNFT) (BWK X 100).

DISCUSSION

Oxidative stress is widely recognized as one of the earliest and most important mechanisms involved in Alzheimer's disease pathogenesis²⁸. In the present study, AlCl₃ administration significantly increased malondialdehyde (MDA) while significantly decreasing the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) ($p < 0.05$). These findings indicate excessive generation of reactive oxygen species (ROS), resulting in membrane lipid peroxidation and exhaustion of endogenous antioxidant defense systems. The observed biochemical alterations are consistent with numerous experimental studies demonstrating that chronic aluminum exposure promotes oxidative injury by disrupting mitochondrial function, increasing free radical production, and impairing antioxidant enzyme activities^{4,29,30}. Elevated MDA reflects severe lipid peroxidation of neuronal membranes, which compromises membrane integrity, synaptic transmission, and neuronal survival, whereas depletion of SOD, CAT, and GPx further exposes neurons to oxidative damage.

Treatment with TT significantly reversed these oxidative changes in a dose-dependent manner. Both doses significantly reduced MDA while restoring SOD, CAT, and GPx activities ($p < 0.05$), with the high-dose TT producing effects comparable to donepezil. These observations suggest that the neuroprotective effect of TT is largely attributable to its potent antioxidant properties^{8,9}.

AlCl₃ markedly increased amyloid- β (A β), phosphorylated tau (pTau), glial fibrillary acidic protein (GFAP), Bax, and Caspase-3 while significantly reducing Bcl-2 expression ($p < 0.05$). These molecular alterations collectively indicate successful induction of Alzheimer's disease-like neuropathology. Increased A β accumulation and tau hyperphosphorylation are the principal pathological hallmarks of Alzheimer's disease and are closely associated with synaptic dysfunction, neuronal degeneration, and progressive cognitive decline³². Similarly, increased GFAP expression reflects astrocyte activation, an important indicator of neuroinflammation and reactive gliosis. The simultaneous increase in Bax and Caspase-3 together with suppression of the anti-apoptotic protein Bcl-2 demonstrates activation of the intrinsic mitochondrial apoptotic pathway, leading to accelerated neuronal death³³.

However, TT significantly attenuated these pathological changes. Both treatment doses significantly reduced A β , pTau, GFAP, Bax, and Caspase-3 while increasing Bcl-2 expression ($p < 0.05$), with greater improvement observed following high-dose treatment. These findings suggest that TT not only suppresses oxidative stress but also interrupts downstream molecular pathways responsible for neuronal degeneration. The increase in Bcl-2 accompanied by reductions in Bax and Caspase-3 indicates inhibition of mitochondrial-mediated apoptosis, thereby promoting neuronal survival. Likewise,

suppression of GFAP suggests attenuation of astrocytic activation and neuroinflammatory responses. The reduction in A β and pTau further implies that TT may interfere with amyloidogenic processing and abnormal tau phosphorylation, thereby limiting progression of Alzheimer's-like pathology.

More so, the behavioral findings strongly support the biochemical evidence. Rats exposed to AlCl₃ exhibited prolonged escape latency in the Morris Water Maze together with marked anxiety-like behavior in the Elevated Plus Maze, evidenced by reduced open-arm exploration, increased closed-arm occupancy, excessive grooming, and impaired rearing activity. These behavioral abnormalities are characteristic manifestations of prefrontal cortical dysfunction³⁴. TT treatment significantly improved spatial learning, memory performance, exploratory behavior, and anxiety indices ($p < 0.05$), with high-dose TT producing improvements comparable to donepezil. These behavioral improvements likely reflect preservation of neuronal integrity within cognitive brain regions following attenuation of oxidative stress and apoptosis. The observed improvements in body weight following TT administration provide additional evidence of its systemic protective effects. Although initial body weights were comparable among groups, AlCl₃-treated rats exhibited significantly reduced final body weight ($p = 0.037$), suggesting generalized toxicity and impaired physiological function. TT-treated rats demonstrated greater weight gain, particularly at the higher dose, indicating improved overall health status. Interestingly, brain weights were not significantly different among groups, suggesting that functional impairment preceded overt anatomical atrophy, which is consistent with the early stages of experimental Alzheimer's disease³⁵.

The histological findings corroborate the biochemical and behavioral results. AlCl₃ exposure produced severe neuronal degeneration characterized by neuronal loss, cytoplasmic vacuolation, pyknotic nuclei, karyorrhexis, disruption of Nissl substance, and abundant senile plaque formation within the prefrontal cortex. These histopathological abnormalities are classical features of Alzheimer's disease and reflect irreversible neuronal injury resulting from oxidative stress, neuroinflammation, and apoptosis^{35,36}. TT treatment markedly preserved neuronal cytoarchitecture, reduced neuronal degeneration, maintained Nissl substance integrity, and decreased amyloid plaque deposition. The preservation of neuronal morphology observed in both H&E and Cresyl Fast Violet staining together with reduced Bielschowsky-positive senile plaques provides compelling morphological evidence supporting the biochemical demonstration of reduced A β accumulation and apoptotic activity.

CONCLUSION

Tetrapleura tetraptera effectively ameliorated AlCl_3 -induced Alzheimer's disease-like pathology through multiple interconnected mechanisms involving suppression of oxidative stress, inhibition of neuronal apoptosis, attenuation of neuroinflammation, reduction of amyloid and tau pathology, preservation of neuronal architecture, and restoration of cognitive function. The convergence of behavioral, biochemical, and histological evidence substantially strengthens the validity of these findings and supports the growing evidence that *Tetrapleura tetraptera* may represent a promising phyto-therapeutic candidate in a dose dependent regimen for the prevention or management of Alzheimer's disease.

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