



# Histomorphological and Biochemical Assessment of the Protective Effects of *Prunus Domestica* Leaf Extract on Aluminium Chloride Induced Hypothalamic Toxicity in Wistar Rats

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## Abstract

## Review Article

**Background:** Aluminum chloride ( $AlCl_3$ ) is a known environmental neurotoxin implicated in oxidative stress and hypothalamic dysfunction. Natural antioxidants from medicinal plants have shown promise in mitigating heavy metal-induced neurotoxicity.

**Objective:** This study evaluated the protective effects of ethanol leaf extract of *Prunus domestica* L. against  $AlCl_3$ -induced hypothalamic toxicity in adult male Wistar rats.

**Methods:** Forty (40) adult male Wistar rats were divided into five groups ( $n = 8$ ): Control,  $AlCl_3$  (100 mg/kg),  $AlCl_3 + P. domestica$  (200 mg/kg),  $AlCl_3 + P. domestica$  (400 mg/kg), and  $AlCl_3 + Vitamin E$  (100 mg/kg). Treatments were administered orally for 28 days. Oxidative stress biomarkers (MDA, SOD, CAT, GSH), hormonal assays (GnRH, LH, FSH, testosterone), and histopathological examinations of the hypothalamus were conducted.

**Results:**  $AlCl_3$  significantly increased MDA levels and decreased antioxidant enzyme activities and hormonal concentrations ( $p < 0.05$ ). Treatment with *P. domestica* extract significantly reversed these alterations in a dose-dependent manner. Histological analysis showed restoration of neuronal architecture in treated groups compared with severe degeneration in the  $AlCl_3$  group.

**Conclusion:** Ethanol leaf extract of *P. domestica* significantly ameliorates aluminum-induced hypothalamic toxicity through antioxidant and neuroendocrine regulatory mechanisms.

**Keywords:** Aluminum chloride, hypothalamus, oxidative stress, *Prunus domestica*, Wistar rats.

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## 1. INTRODUCTION

Contact with heavy metals in the environment poses a major threat to public health, potentially triggering long-term harm in both humans and wildlife.

Aluminum ranks among the most prevalent elements in the planet's crust and finds extensive application in manufacturing, medications, and household items. Once thought to be benign, recent research indicates



that prolonged aluminum contact could play a role in degenerative brain conditions and hormonal imbalances (Bondy, 2010).

AlCl<sub>3</sub> serves as a common model for inducing neurotoxicity in experiments, as it easily penetrates the blood-brain barrier and builds up in neural tissues. Upon accumulation, aluminum ions stimulate the formation of reactive oxygen species (ROS), resulting in oxidative imbalance, impaired mitochondrial activity, membrane lipid breakdown, and cell death in neurons (Verstraeten et al., 2008). Such mechanisms are linked to memory deficits and progressive neural decline.

The hypothalamus is vital for regulating bodily equilibrium, overseeing hormonal and neural responses. It acts as the primary controller of the hypothalamic-pituitary-gonadal (HPG) pathway, managing fertility through gonadotropin-releasing hormone (GnRH) release (Saper & Lowell, 2014). Compromise to hypothalamic cells can thus cause endocrine irregularities, metabolic issues, and fertility problems.

Oxidative imbalance emerges as a core driver of aluminum-related neural harm. Overproduction of ROS overwhelms innate protective systems, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), leading to cellular injury. As a result, interventions that bolster these defenses could shield against aluminum effects (Butterfield & Halliwell, 2019).

Herbal remedies offer valuable reservoirs of natural protective agents. Various plant-derived chemicals, such as flavonoids, phenolic compounds, tannins, and pigments, display robust capacity to neutralize free radicals and reduce oxidative harm (Fraga et al., 2010). *Prunus domestica* L. (commonly known as plum) stands out for its abundant bioactive profile and health-promoting traits.

Prior investigations highlight that *P. domestica* harbors elevated levels of polyphenolic substances and flavonoids with strong protective capabilities. These elements have proven effective in safeguarding cells from oxidative and inflammatory stress. Nevertheless, data on the neural shielding properties of *P. domestica* leaves, especially

concerning aluminum-triggered hypothalamic harm, remain scarce (Gil et al., 2002; Oszmiański & Wojdyło, 2005).

Hence, this research sought to explore the tissue-level and biochemical safeguarding influences of ethanolic *P. domestica* leaf extract on AlCl<sub>3</sub>-provoked hypothalamic injury in Wistar rats.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

Forty adults' male Wistar rats weighing between 180g – 220g were housed under standard laboratory conditions with ad libitum access to feed and water.

### ETHICAL APPROVAL

The ethical approval was obtained from the institutional ethical committee with Ethical approval number: FUTO/ANA/REC/2026/024.

### 2.2 Preparation of Extract

The collected leaves were washed thoroughly under running water to remove dirt and debris after which it was rinsed in distilled water. The leaves were air-dried at room temperature under shade to prevent degradation of active compounds. The dried leaves were pulverized into fine powder. A known weight of the powdered plant material was soaked in 2.5 liters of 70% ethanol. The mixture was kept in a sealed glass container for 72 hours with intermittent shaking to ensure proper extraction. The mixture was filtered using a muslin cloth followed by Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator at 40 to 50°C. The dried extract was weighed to determine percentage yield and stored in a refrigerator at 4°C until use.

### 2.3 Plant collections

Fresh leaves of *Prunus domestica* were collected from a natural habitat in Ihiagwa, in Imo State. The plant was identified and authenticated by Nwankwo, E. O. in the department of Botany, Federal University of Technology, Owerri, Imo State with a voucher serial number FUTO/HERH/PD/2026/028.

**Table 1: Showing Experimental Design**

Group	Administration	Number of rats	Duration (Weeks)
Control A	Feed + water	8	4
B	Feed + water + AlCl <sub>3</sub> (100 mg/kg)	8	4
C	Feed + water + AlCl <sub>3</sub> + P. domestica (200 mg/kg)	8	4
D	Feed + water + AlCl <sub>3</sub> + P. domestica (400 mg/kg)	8	4
E	Feed + water + AlCl <sub>3</sub> + Vitamin E (100 mg/kg)	8	4

n = 40

## 2.5 Biochemical Assays

Hypothalamic samples were blended in chilled phosphate solution and spun to yield supernatant for testing.

## 2.6 Lipid Peroxidation

Assessment of lipid breakdown involved quantifying malondialdehyde (MDA) via the thiobarbituric acid reactive substances approach.

## 2.7 Superoxide Dismutase

SOD function was gauged by its ability to halt the self-oxidation of epinephrine.

## 2.7 Catalase

Catalase performance was determined through the pace of hydrogen peroxide breakdown.

## 2.9 Reduced Glutathione

GSH quantities were quantified employing Ellman's reagent.

## 2.10 Histopathology

Hypothalamic tissues were fixed in 10% neutral buffered formalin for 48 hours. The tissues were dehydrated in graded alcohol, cleared with xylene, and embedded in paraffin wax. Sections of approximately 5 µm were cut and stained with hematoxylin and eosin.

Microscopic examination was performed using a light microscope to assess neuronal morphology and structural integrity.

## 2.11 Statistical Analysis

Findings are presented as mean ± standard error of the mean (SEM). Variance was examined via one-way ANOVA, succeeded by Tukey's multiple comparison test. Significance was set at p<0.05.

3. RESULTS

**Table 2: Showing Oxidative Stress Markers**

Group	Control (Feed + water)	Feed + water + AlCl <sub>3</sub> (100mg/kg)	Feed + water + AlCl <sub>3</sub> (100mg/kg) + 200 mg/kg extract	Feed + water + AlCl <sub>3</sub> (100mg/kg) + 400 mg/kg extract	Feed + water + AlCl <sub>3</sub> (100mg/kg) + Vit E
MDA	1.25±0.10	3.90±0.20*	2.40±0.15#	1.60±0.12#	1.50±0.11#
SOD	8.50±0.32	4.10±0.25*	6.80±0.28#	7.90±0.30#	8.20±0.29#
CAT	12.30±0.45	6.50±0.30*	9.20±0.40#	11.40±0.38#	11.90±0.41#
GSH	6.20±0.21	2.80±0.18*	4.50±0.20#	5.80±0.25#	6.00±0.23#

\*P<0.05 vs control; #P<0.05 vs AlCl<sub>3</sub>

**Table 3: Showing Hormonal Profile**

Group	Control (Feed + water)	Feed + water + AlCl <sub>3</sub> (100mg/kg)	Feed + water + AlCl <sub>3</sub> (100mg/kg) + 200 mg/kg extract	Feed + water + AlCl <sub>3</sub> (100mg/kg) + 400 mg/kg extract	Feed + water + AlCl <sub>3</sub> (100mg/kg) + Vit E
GnRH	5.80±0.30	2.10±0.15*	4.10±0.25#	5.20±0.28#	5.50±0.29#
LH	6.50±0.35	3.00±0.20*	5.00±0.30#	6.00±0.32#	6.20±0.34#
FSH	5.90±0.28	2.80±0.18*	4.60±0.22#	5.40±0.25#	5.70±0.27#
Testosterone	4.20±0.20	1.60±0.12*	3.10±0.18#	3.90±0.21#	4.00±0.22#

### 3.2 Histological Findings:

Group A (Feed + water only): Photomicrograph (X100/X400, H&E) reveals normal hypothalamic section with vibrant granular neurons (NC).

Group B ( $AlCl_3$  100 mg/kg): Photomicrograph (X100/X400, H&E) displays hypothalamic section with moderate vacuoles (V) and neuronal loss.

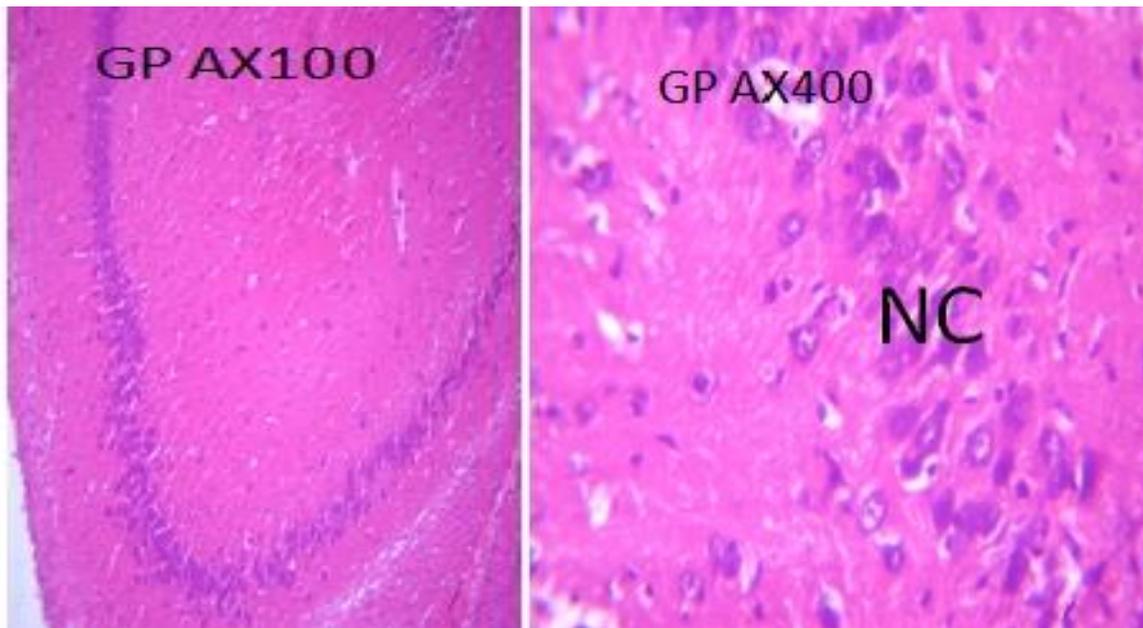
Group C ( $AlCl_3$  + *P. domestica* 200 mg/kg): Photomicrograph (X100/X400, H&E) shows hypothalamic section with partial recovery, featuring

slight microcystic areas (MS) and defined pyramidal neurons (PC).

Group D ( $AlCl_3$  + *P. domestica* 400 mg/kg): Photomicrograph (X100/X400, H&E) indicates hypothalamic section with gentle recovery and moderate vacuoles (V) in neurons (N).

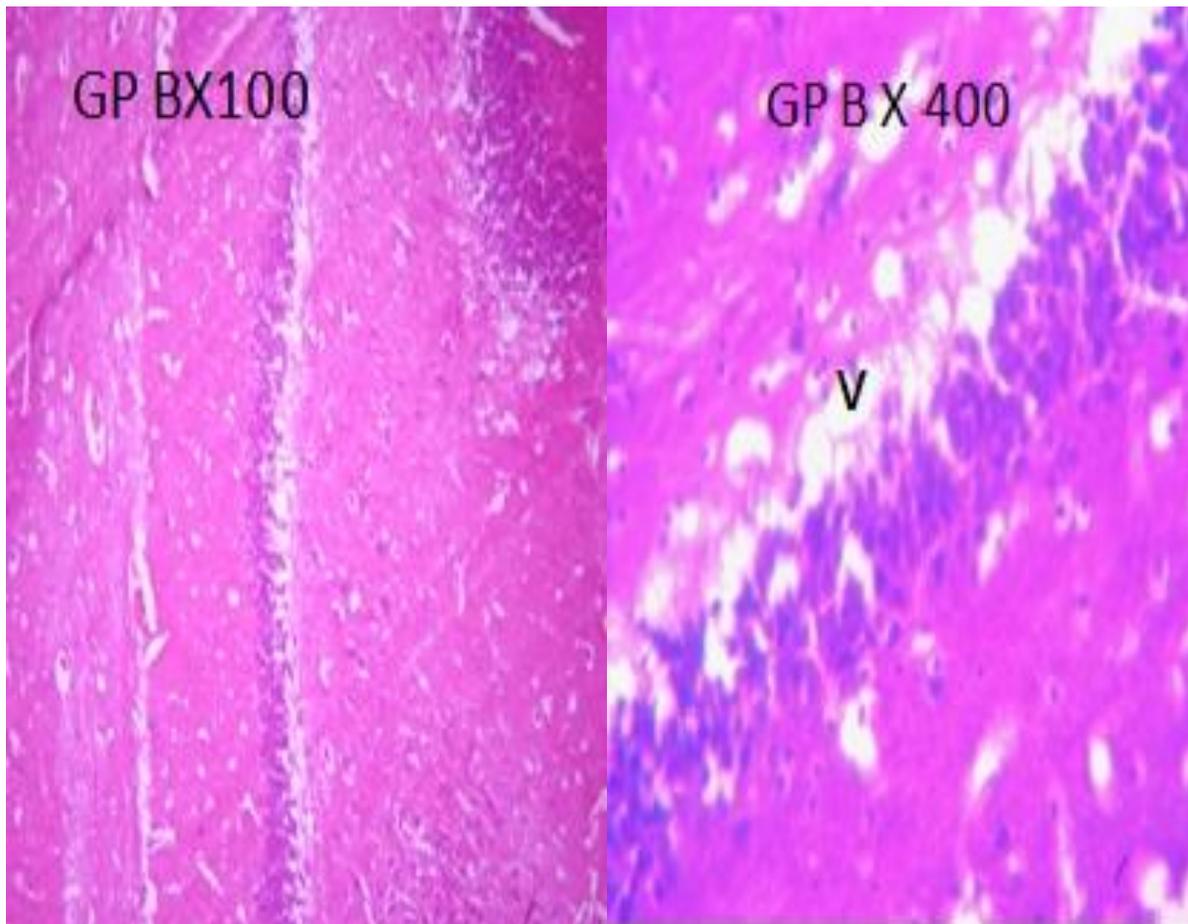
Group E ( $AlCl_3$  + Vitamin E 100 mg/kg): Photomicrograph (X100/X400, H&E) depicts hypothalamic section with intermediate recovery and functional neurons (NC).

#### Group A (Feed + water only)



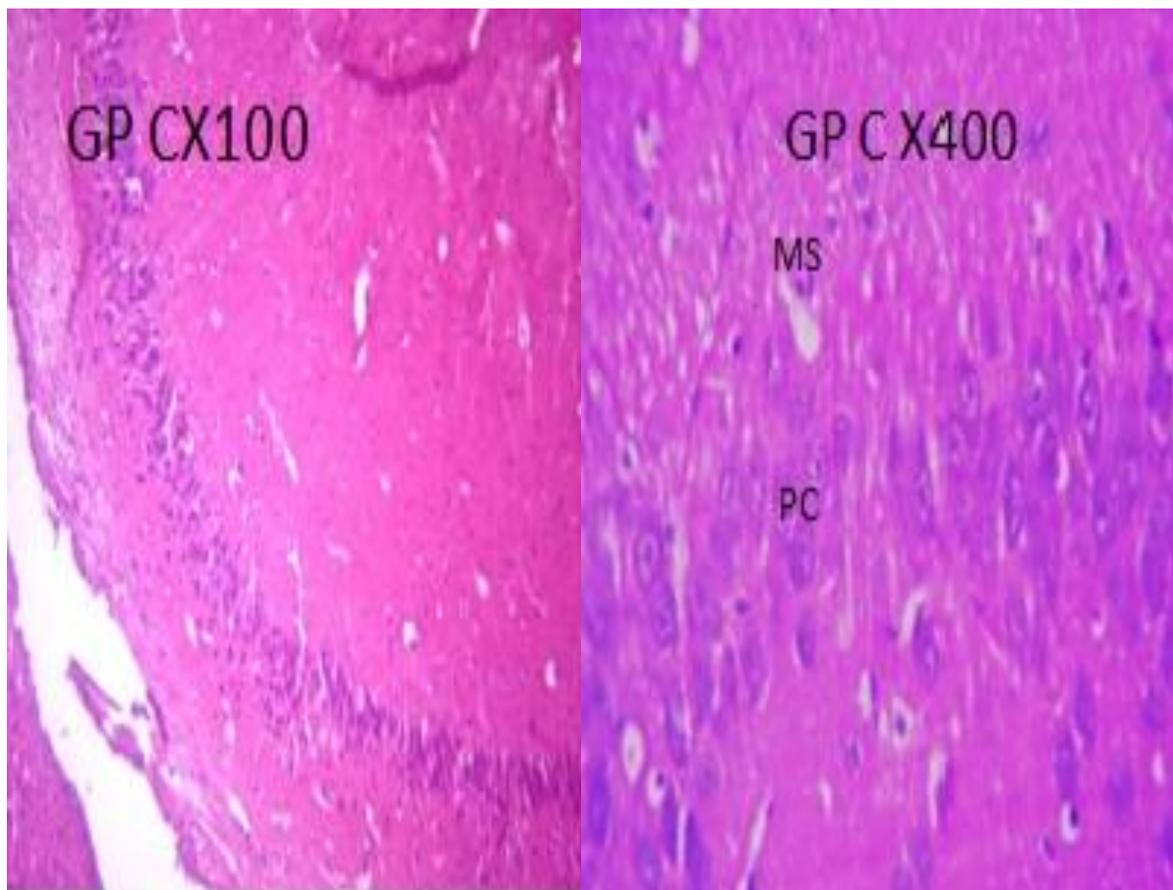
**Plate 1:** Photomicrograph (X100/X400 (H/E) of group A (normal control) section of hypothalamus shows active granular cells (NC)

Group B ( $AlCl_3$  + *P. domestica* (100 mg/kg)



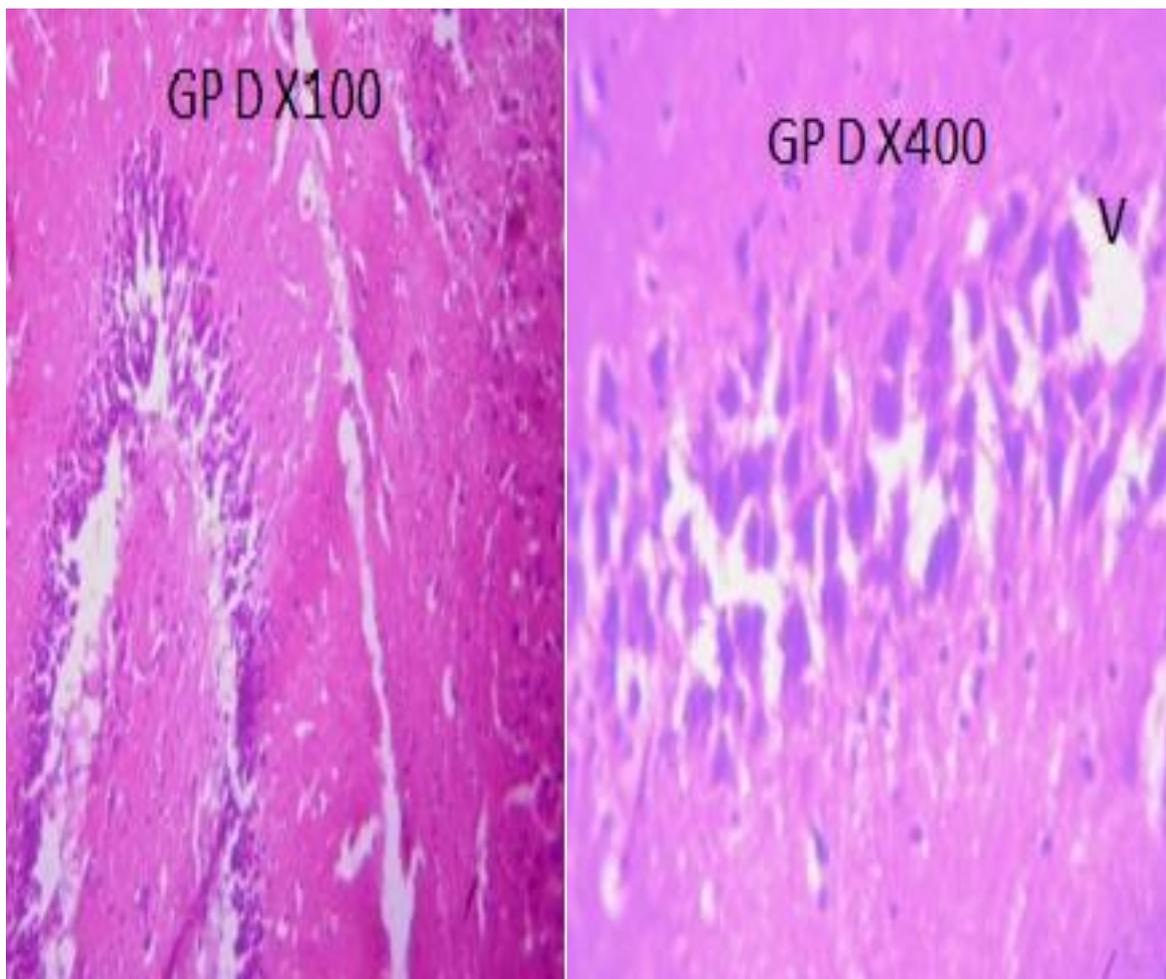
**Plate 2:** Photomicrograph (X100/X400(H/E) of group B (negative control) section of the hypothalamus exposed to Aluminium chloride shows moderate vacuolation (V) with loss of neuronal cells.

**Group C (AlCl<sub>3</sub> + *P. domestica* (200 mg/kg))**



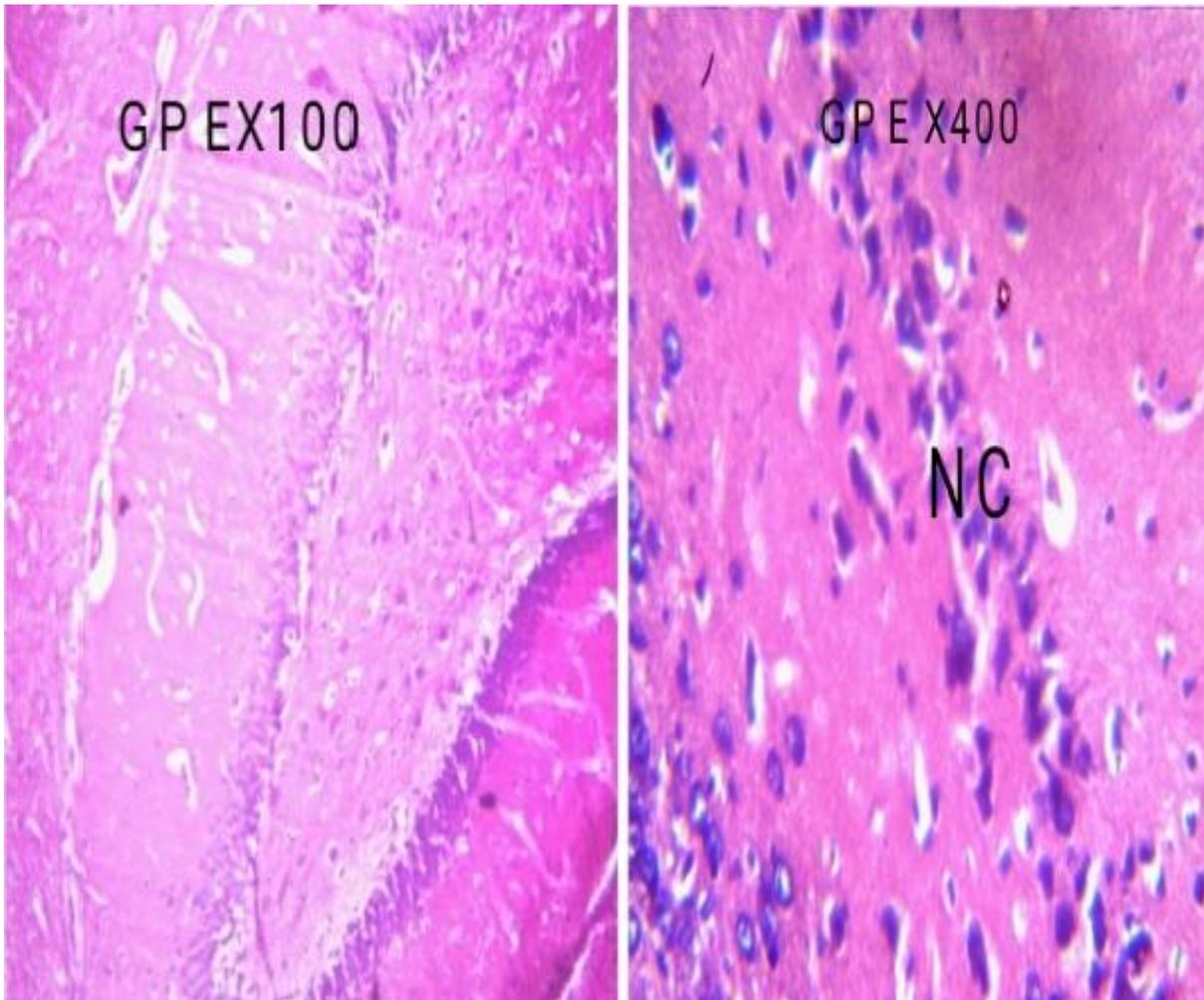
**Plate 3:** Photomicrograph (X100/X400(H/E)) of group C section of the hypothalamus exposed to Aluminium chloride and subsequently treated with 200mg/kg of *Prunus domestica* extract shows moderate healing with mild microcystic space (MS) and well outlined Pyramidal cell (PC).

Group D ( $AlCl_3$  + *P. domestica* (400 mg/kg))



**Plate 4:** Photomicrograph (X100/X400(H/E) of group D section of the hypothalamus induced with Aluminium chloride and treated with 400mg/kg of *Prunus domestica* extract shows mild healing with moderate vacuolation (V) of neuronal cell (N).

**Group E (AlCl<sub>3</sub> + Vitamin E (100 mg/kg))**



**Plate 5:** Photomicrograph (X100/X400(H/E)) of group E section of the hypothalamus exposed to Aluminium chloride and subsequently treated with 100mg/kg of Vitamin E (Tocopherol) shows moderate healing with active neuronal cell (NC).

#### 4. DISCUSSION

This investigation illustrates that AlCl<sub>3</sub> exposure provokes notable oxidative burden and architectural disruption in hypothalamic cells. Heightened MDA values in the aluminum cohort signify amplified lipid degradation from excessive ROS production. Comparable outcomes appear in earlier explorations

of aluminum neural toxicity (Bondy, 2010; Yokel, 2013).

Declines in protective enzymes like SOD, CAT, and GSH point to weakened internal safeguards. These components are essential for neutralizing ROS and upholding cell equilibrium. Suppression renders

brain tissues prone to harm (Nampoothiri et al., 2015; Kumar et al., 2009).

Endocrine evaluations showed substantial drops in GnRH, LH, FSH, and testosterone post-aluminum treatment, suggesting interference with the HPG pathway critical for fertility control. Past work confirms heavy metals can disrupt hormonal signals and fertility (Jobling et al., 1995; Sharma & Mishra, 2006).

Administration of *P. domestica* leaf extract notably mitigated these shifts. It lowered lipid degradation and boosted enzyme functions, likely owing to flavonoid and phenolic free-radical neutralizers. Recent studies on *P. domestica* extracts corroborate these antioxidative and memory-enhancing benefits in aging models (Saha et al., 2011; Auddy et al., 2003; Liu et al., 2012; Kawahara & Kato-Nishimura, 2011).

Microscopic observations reinforced biochemical data. Degeneration and voids in aluminum-exposed rats lessened in extract-treated animals, implying preservation of cell wholeness and aversion of neuron loss (Zhu et al., 2006).

In totality, outcomes imply *P. domestica* leaf extract delivers substantial neural protection via antioxidative and endocrine oversight. To deepen insights, future probes could explore molecular routes, such as apoptosis indicators or inflammation markers, for comprehensive mechanisms (Exley, 2013).

## 5. Limitations

This study employed a modest cohort size (n=8/group), which suffices for initial findings but may restrict power for subtle effects. Exclusive use of male rats overlooks possible gender variances in HPG responses; incorporating females in subsequent research would broaden applicability. The elevated AlCl<sub>3</sub> dosage (100 mg/kg) exceeds typical environmental levels, so lower doses could better simulate real scenarios. Long-term impacts or recovery phases were not examined, representing avenues for expansion.

## 6. Conclusion

Outcomes from this inquiry affirm that ethanolic *P. domestica* leaf extract confers meaningful defense against AlCl<sub>3</sub>-elicited hypothalamic harm in Wistar rats. The extract proficiently lessens oxidative burden, augments protective enzyme operations, reinstates endocrine equilibrium, and safeguards hypothalamic neuron layout. These insights propose *P. domestica* leaves hold viable neural protective promise as a herbal remedy for heavy metal neural toxicity. While rat-based, cautious extrapolation to humans warrants further clinical validation (Verstraeten et al., 2008).

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