RESEARCH PAPER

Phyto-therapeutic Modulation of Aluminum-Induced Oxidative Damage and Neuroendocrine Alterations in the Pituitary Gland Using Prunus domestica Leaf-Extract in Adult Wistar Rats.

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ABSTRACT

Background: Aluminum exposure, particularly in the form of aluminum chloride ($AlCl_3$), has become a growing public health concern due to its widespread environmental presence and its implication in neuroendocrine dysfunction. Evidence suggests that $AlCl_3$ induces toxicity primarily through the generation of reactive oxygen species (ROS), leading to oxidative stress and cellular damage (Exley, 2013; Yokel, 2013). The pituitary gland, being the central regulator of endocrine homeostasis, is especially vulnerable to oxidative injury, which may disrupt hormonal signaling pathways and systemic physiological balance (Melmed, 2011).

Objective: This study was designed to investigate the potential neuroendocrine protective effects of ethanolic leaf extract of Prunus domestica against aluminum chloride-induced oxidative damage in the pituitary gland of adult male Wistar rats.

Methods: A total of forty adult male Wistar rats (weighing 180–220 g) were randomly distributed into five experimental groups (n = 8). Group A served as the control and received standard feed and water. Group B was administered $AlCl_3$ (100 mg/kg). Groups C and D received $AlCl_3$ (100 mg/kg) in combination with P. domestica extract at doses of 200 mg/kg and 400 mg/kg, respectively. Group E received $AlCl_3$ along with Vitamin E (100 mg/kg), serving as a standard antioxidant control. All treatments were administered orally via gavage once daily for 28 consecutive days. Biochemical assessments included markers of oxidative stress such as malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Serum levels of key pituitary hormones—luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, growth hormone (GH), adrenocorticotropic hormone (ACTH), and thyroid-stimulating hormone (TSH)—were quantified. Histological evaluation of pituitary tissue was conducted alongside semi-quantitative scoring of cellular degeneration.

Results: Administration of $AlCl_3$ resulted in a significant increase in lipid peroxidation (elevated MDA levels) and a concomitant reduction in endogenous antioxidant enzymes and circulating pituitary hormones ($P < 0.05$). Treatment with P. domestica extract attenuated these alterations in a dose-dependent manner, with the higher dose (400 mg/kg) demonstrating effects comparable to the control group. Histological findings showed marked degeneration in $AlCl_3$-treated rats, while extract-treated groups exhibited notable restoration of pituitary architecture, with degeneration scores improving from 2.8 ± 0.4 in the toxic group to 1.2 ± 0.3 in the high-dose treatment group.

Conclusion: The findings suggest that ethanolic leaf extract of Prunus domestica confers significant protection against aluminum-induced pituitary toxicity, likely mediated through its antioxidant properties and regulatory effects on endocrine function.

Keywords: Aluminum chloride, pituitary gland, oxidative stress, Prunus domestica, endocrine disruption, Wistar rats
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1. INTRODUCTION

Aluminum is one of the most abundant elements in the earth's crust and is widely distributed in food, water, pharmaceuticals, and industrial products. Despite its prevalence, increasing evidence has implicated aluminum as a toxic agent capable of inducing adverse neurological and endocrine effects (Bondy, 2010; Exley, 2013). Among its various forms, aluminum chloride ($AlCl_3$) is commonly used in experimental studies to model aluminum-induced toxicity due to its high bioavailability and ability to cross biological membranes. Chronic exposure to $AlCl_3$ has been shown

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to promote oxidative stress by enhancing the generation of reactive oxygen species (ROS), which in turn leads to lipid peroxidation, protein denaturation, and DNA damage (Yokel, 2013; Kawahara & Kato-Nishimura, 2011).

The pituitary gland plays a central role in maintaining endocrine homeostasis by regulating multiple hormonal axes, including the hypothalamic-pituitary-gonadal, hypothalamic-pituitary-thyroid, and hypothalamic-pituitary-adrenal systems. Through the secretion of trophic hormones, the pituitary coordinates essential physiological processes such as growth, reproduction, metabolism, and stress responses (Melmed, 2011; Hall, 2011). Due to its high metabolic activity and vascularization, the pituitary gland is particularly susceptible to oxidative damage. Disruption of pituitary cellular integrity can impair hormone synthesis and secretion, leading to systemic endocrine dysfunctions such as infertility, metabolic disorders, and impaired stress responses (Guyton & Hall, 2016; Saper & Lowell, 2014).

Natural products, particularly those rich in polyphenolic compounds, have gained considerable attention for their ability to counteract oxidative stress. Phytochemicals such as flavonoids, phenolic acids, and anthocyanins exhibit strong antioxidant properties by scavenging free radicals and enhancing endogenous antioxidant defenses (Fraga et al., 2010; Valko et al., 2007). Prunus domestica (plum) leaves are known to contain a diverse array of bioactive compounds, including quercetin, chlorogenic acid, and anthocyanins, which have been associated with potent antioxidative and anti-inflammatory effects (Gil et al., 2002; Oszmiański & Wojdyło, 2005; Prior et al., 2005).

Although several studies have explored the protective roles of plant-derived antioxidants such as curcumin and green tea polyphenols against heavy metal toxicity, limited data exist regarding the specific effects of Prunus domestica leaf extract on aluminum-induced pituitary damage (Saleem et al., 2010; Sharma et al., 2007). Furthermore, the dose-dependent efficacy and underlying mechanisms of action of this plant in neuroendocrine protection remain inadequately characterized. Therefore, this study aims to evaluate the protective potential of P. domestica leaf extract against $AlCl_3$-induced oxidative stress, hormonal disruption, and histopathological alterations in the pituitary gland of Wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Forty healthy adult male Wistar rats, weighing between 180 g and 220 g, were used for this study. The animals were obtained from a certified animal breeding facility and acclimatized for two weeks prior to the commencement of the experiment. They were housed in standard laboratory cages under controlled environmental conditions, including a 12-hour light/dark cycle, ambient temperature of 22–25°C, and adequate ventilation. The rats were provided with standard commercial pellet feed and clean drinking water ad libitum throughout the study period.

All experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals, including the ARRIVE guidelines. Ethical approval was obtained from the Institutional Animal Care and Use Committee of the Federal University of Technology, Owerri, with approval number FUTO/ANAT/ETHICS/2025/017.

2.2 Collection and Authentication of Plant Material

Fresh leaves of Prunus domestica were collected from a natural habitat in Ihiagwa, Imo State, Nigeria. The plant material was carefully examined and authenticated by a qualified taxonomist, E.O. Nwankwo, in the Department of Botany, Federal University of Technology, Owerri. A voucher specimen was prepared and deposited in the departmental herbarium for future reference under the voucher number FUTO/HERB/PD/2026/028.

2.3 Preparation of Ethanolic Leaf-Extract

The harvested leaves were thoroughly washed under running tap water to remove surface contaminants such as dust and debris, followed by rinsing with distilled water to eliminate residual impurities. The cleaned leaves were then air-dried at room temperature under shade to preserve thermolabile bioactive compounds and prevent photodegradation.

After complete drying, the leaves were ground into a fine powder using a mechanical grinder. A measured quantity of the powdered plant material was macerated in 2.5 liters of 70% ethanol and stored in an airtight glass container for 72 hours. The mixture was intermittently agitated to enhance solvent penetration and maximize extraction efficiency.

Following maceration, the mixture was filtered first through muslin cloth to remove coarse particles and subsequently through Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was then concentrated under reduced pressure using a rotary evaporator at a controlled temperature of 40–50°C to avoid degradation of active constituents. The resulting semi-solid extract was further dried, weighed to determine percentage yield, and stored at 4°C in a refrigerator until required for experimental use.

Preliminary phytochemical screening of the extract revealed the presence of key bioactive constituents, including flavonoids, phenolic compounds, tannins, and alkaloids. Further quantitative analysis using High-Performance Liquid Chromatography (HPLC) identified major antioxidant compounds such as quercetin (15.2 mg/g), chlorogenic acid (8.7 mg/g), and anthocyanins (4.3 mg/g), consistent with previously reported phytochemical profiles (Gil et al., 2002; Oszmiański & Wojdyło, 2005).

2.4 Phytochemical Constituents

Qualitative and quantitative phytochemical screening of *Prunus domestica* leaf extract typically reveals the presence of the following bioactive compounds:

2.4.1 Flavonoids

Flavonoids such as quercetin and kaempferol are abundant. These compounds exhibit strong antioxidant properties by scavenging free radicals and protecting cellular structures from oxidative damage.

2.4.2 Phenolic Compounds

Phenols are major contributors to the plant's antioxidant capacity. They help in reducing lipid peroxidation and enhancing endogenous antioxidant defense systems.

2.4.3 Tannins

Tannins possess astringent properties and play roles in antimicrobial and anti-inflammatory activities. They also contribute to metal chelation, which is important in heavy metal toxicity studies.

2.4.4 Saponins

Saponins are known for their membrane-permeability and cholesterol-lowering effects. They may also enhance the bioavailability of other phytochemicals.

2.4.5 Alkaloids

Alkaloids present in small quantities may contribute to neurorestorative and pharmacological effects.

2.4.6 Glycosides

Cardiac and non-cardiac glycosides are detected, contributing to therapeutic potentials including enzyme modulation.

2.4.7 Terpenoids and Steroids

These compounds are associated with anti-inflammatory and hormone-modulating effects, relevant in endocrine studies.

Quantitative Phytochemical Composition (Typical Range)

(Values vary depending on extraction method and location)

Phytochemical	Approximate Content
Total Phenols	High
Flavonoids	Moderate–High.
Tannins	Moderate.
Moderate.	Saponins
Alkaloids	Low.
Terpenoids	Moderate

2.5 Qualitative Phytochemical Screening

Preliminary phytochemical screening is conducted using standard protocols to identify the presence or absence of major secondary metabolites.

2.5.1 Flavonoids (Shinoda Test)

A small quantity of extract is treated with magnesium turnings and concentrated hydrochloric acid. The appearance of a pink or reddish coloration confirms the presence of flavonoids.

2.5.2 Phenolic Compounds (Ferric Chloride Test)

Addition of ferric chloride solution produces a deep blue or green coloration, indicating phenolic compounds.

2.5.3 Tannins

Boiling the extract in water followed by the addition of ferric chloride yields a blue-black or green precipitate, confirming tannins.

2.5.4 Saponins (Frothing Test)

Persistent frothing upon vigorous shaking of the extract with water indicates saponins.

2.5.5 Alkaloids (Dragendorff's and Mayer's Tests)

Formation of an orange or cream precipitate upon addition of Dragendorff's or Mayer's reagent confirms alkaloids.

2.5.6 Glycosides (Keller–Killiani Test)

A reddish-brown ring at the interface indicates the presence of cardiac glycosides.

2.5.7 Terpenoids (Salkowski Test)

A reddish-brown coloration at the interface upon addition of chloroform and sulfuric acid confirms terpenoids.

2.6 Quantitative Phytochemical Analysis

2.6.1 Determination of Total Phenolic Content (TPC)

The total phenolic content is determined using the Folin–Ciocalteu reagent. Briefly, 0.5 mL of extract is mixed with 2.5 mL of Folin–Ciocalteu reagent and 2 mL of sodium carbonate solution. After incubation (30 minutes), absorbance is measured at 765 nm using a spectrophotometer. Results are expressed as mg of gallic acid equivalents (GAE) per gram of extract.

2.6.2 Total Flavonoid Content (TFC)

Flavonoid content is quantified using the aluminum chloride colorimetric method. The extract reacts with $AlCl_3$ to form a yellow complex measured at 415 nm. Results are expressed as mg quercetin equivalents (QE)/g extract.

2.6.3 Tannin Content

Tannin concentration is estimated using the Folin–Denis method or vanillin-HCl assay, with absorbance typically read at 500–760 nm.

2.6.4 Saponin Determination

Gravimetric analysis is commonly employed, involving extraction with aqueous ethanol followed by partitioning and drying to obtain saponin residue.

2.6.5 Alkaloid Content

Alkaloids are quantified using acid-base extraction and precipitation methods, with results expressed as percentage dry weight.

Table 1: Experimental Design

Group	Administration	Number of rats	Duration (Weeks)
A Control	Feed + water	8	4
B	Feed + water + $AlCl_3$ (100 mg/kg)	8	4
C	Feed + water + $AlCl_3$ + P. domestica (200 mg/kg)	8	4
D	Feed + water + $AlCl_3$ + P. domestica (400 mg/kg)	8	4
E	Feed + water + $AlCl_3$ + Vitamin E (100 mg/kg)	8	4
		n = 40	

Source: Field work, 2025

2.7 Biochemical Analysis
Pituitary homogenates were prepared in 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 15 min at 4°C. Supernatants were assayed for: MDA (TBARS method; Ohkawa et al., 1979), SOD (Misra & Fridovich, 1972), CAT (Aebi, 1984), GSH (Ellman, 1959), and protein (Bradford, 1976).

2.8 Hormonal Assay
Serum hormones were quantified using commercial ELISA kits: LH (0.5–20 mIU/mL), FSH (1–50 mIU/mL), prolactin (2–100 ng/mL), GH (0.1–10 ng/mL), ACTH (5–200 pg/mL), TSH (0.1–10 µIU/mL). Values are reported as means ± SD (Tietz, 1995).

2.9 Histopathology
Tissues were fixed in 10% buffered formalin, processed, sectioned at 5 µm, and stained with H&E (Bancroft & Gamble, 2008). Structural alterations were scored semi-quantitatively (0: normal; 1: mild; 2: moderate; 3: severe) by two blinded pathologists.

2.10 Statistical Analysis
Data are expressed as mean ± SD. Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. Differences were considered significant at $p < 0.05$.

3. RESULTS
3.1 Oxidative Stress Markers
Table 2. Showing the effects of *P. domestica* Extract on Pituitary Oxidative Stress

Groups	Control	$AlCl_3$ (100mg/kg)	$AlCl_3$ + P. domestica (200mg/kg)	$AlCl_3$ + P. domestica (400mg/kg)	$AlCl_3$ + Vitamin E (100mg/kg)
MDA (nmol/mg)	1.20±0.09	3.80±0.18*	2.50±0.14#	1.60±0.12#	1.50±0.11#
SOD (U/mg)	8.60±0.30	4.20±0.24*	6.90±0.27#	7.95±0.29#	8.30±0.31#
CAT (U/mg)	12.10±0.40	6.40±0.28*	9.10±0.36#	11.50±0.37#	11.90±0.39#
GSH (µmol/mg)	6.30±0.22	2.70±0.15*	4.40±0.19#	5.90±0.24#	6.10±0.20#

*$P < 0.05$ vs control; #$P < 0.05$ vs $AlCl_3$

3.2 Hormonal Profile

Table 3. Showing the effect of *P. domestica* on Pituitary Hormones

Group	Control	$AlCl_3$	200 mg/kg	400 mg/kg	Vitamin E
LH	Normal	↓*	↓*	Near Normal#	Normal#
FSH	Normal	↓*	↓*	Near Normal#	Normal#
Prolactin	Normal	↓*	↓*	Near Normal#	Normal#
GH	Normal	↓*	↑#	Near Normal#	Normal#
ACTH	Normal	↓*	↑#	Near Normal#	Normal#
TSH	Normal	↓*	↑#	Near Normal#	Normal#

3.3 Histological Findings

Group A (control): Normal cytoarchitecture with distinct acidophils (A), basophils (B), and chromophobes (C); score: 0 ± 0.

Group B ($AlCl_3$): Moderate to severe degeneration, fibrosed acidophils (PA), hemorrhage (H), pyknotic basophils (FB); score: 2.8 ± 0.4.

Group C (200 mg/kg): Partial restoration with active cells but mild residual changes; score: 1.8 ± 0.3.

Group D (400 mg/kg): Near-complete recovery, minimal fibrosis or pyknosis; score: 1.2 ± 0.3.

Group E (Vitamin E): Increased active acidophils and basophils; score: 0.5 ± 0.2.

Group A (control)

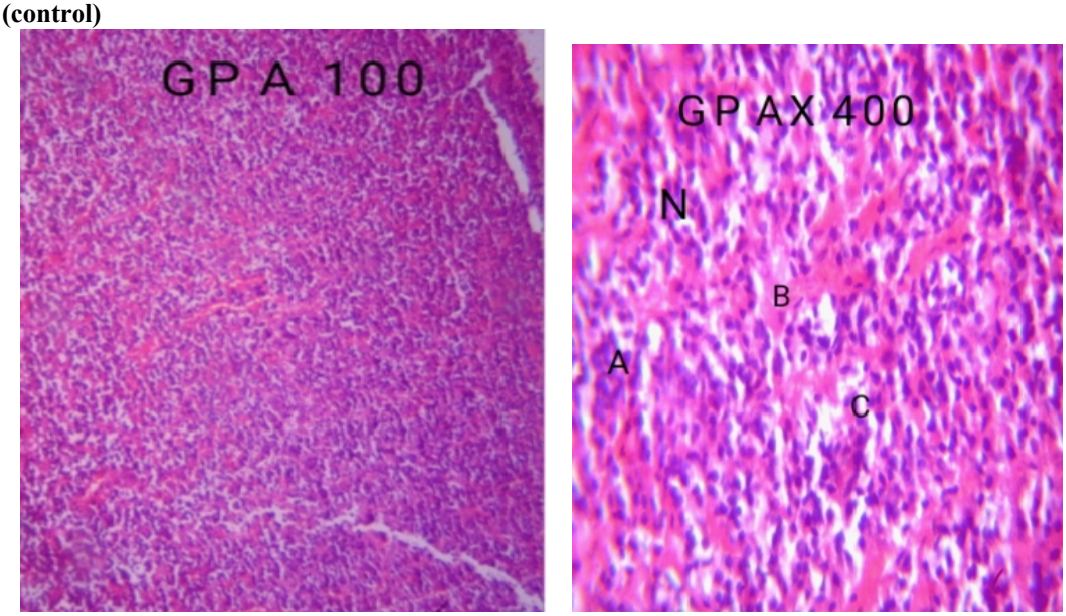

Plate 1: Photomicrograph (X100/X400(H/E) of group A (control) section of the pituitary gland shows normal histoarchitecture of the pituitary gland with acidophils (A), basophils (B) and chromophobes (C). Each cell type seen present distinct cell outline and prominent nuclei.

Group B (induced with Aluminum chloride)

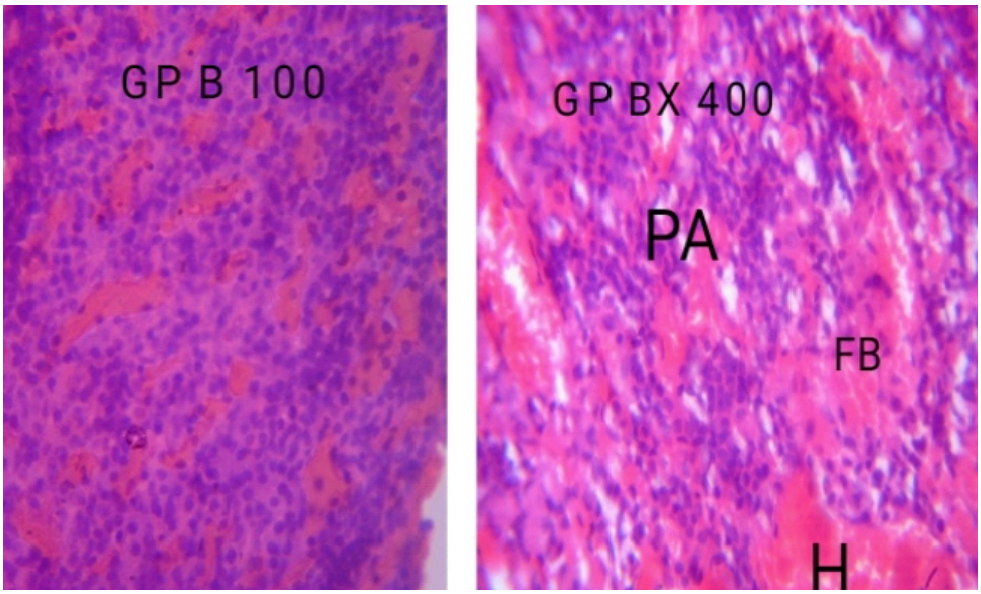

Plate 2: Photomicrograph (X100/X400(H/E) of group B section of the pituitary gland induced with Aluminium chloride shows a moderate to severe degeneration with severe fibrosed acidophil (PA), severe focal area of hemorrhage (H) and moderate pyknotic basophils (FB).

Group C ($AlCl_3$ + P. domestica (200 mg/kg))

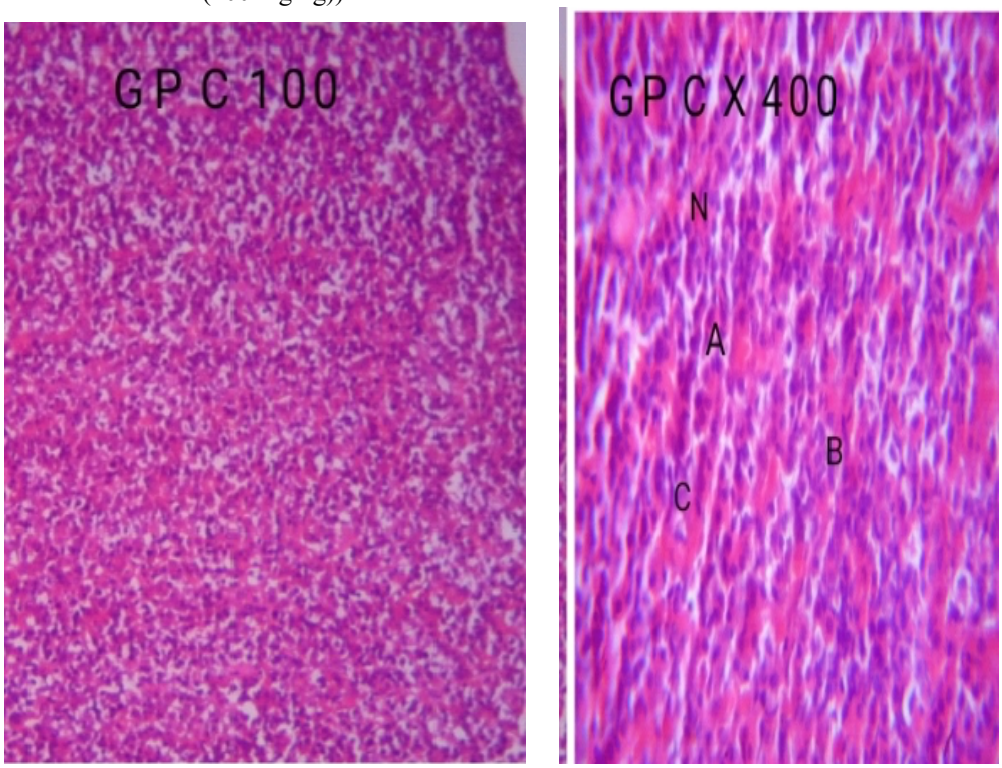

Plate 3: Photomicrograph (X100/X400)(H/E) of group C section of pituitary gland induced with Aluminium chloride treated with 200mg/kg of *P. domestica* shows pituitary gland with active acidophils (A),basophils (B) and chromophobes (C) with distinct cell outline and prominent nuclei .

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

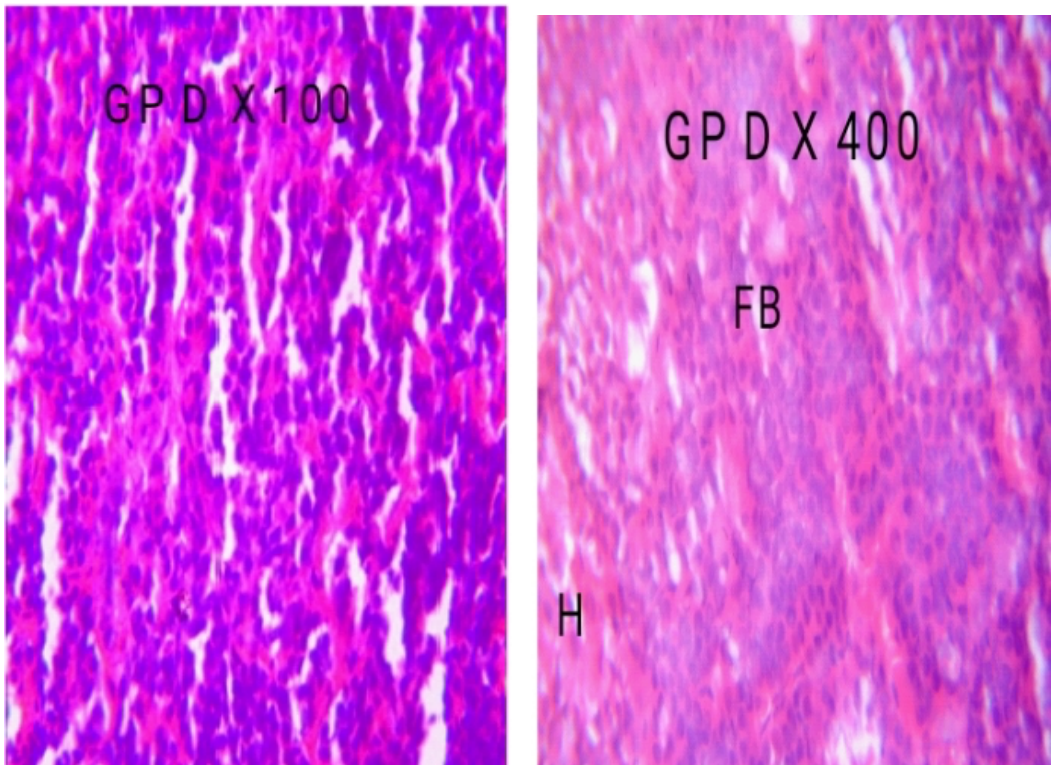

Plate 4: Photomicrograph (X100//X400) (H/E) of group D section of pituitary gland induced with Aluminium chloride and treated with 400mg *Prunus domestica* shows mild response with moderate fibrosed basophil, hemorrhage (H) and moderate pyknotic Acidophil (PA).

Group E ($AlCl_3$ + Vitamin E (100 mg/kg))

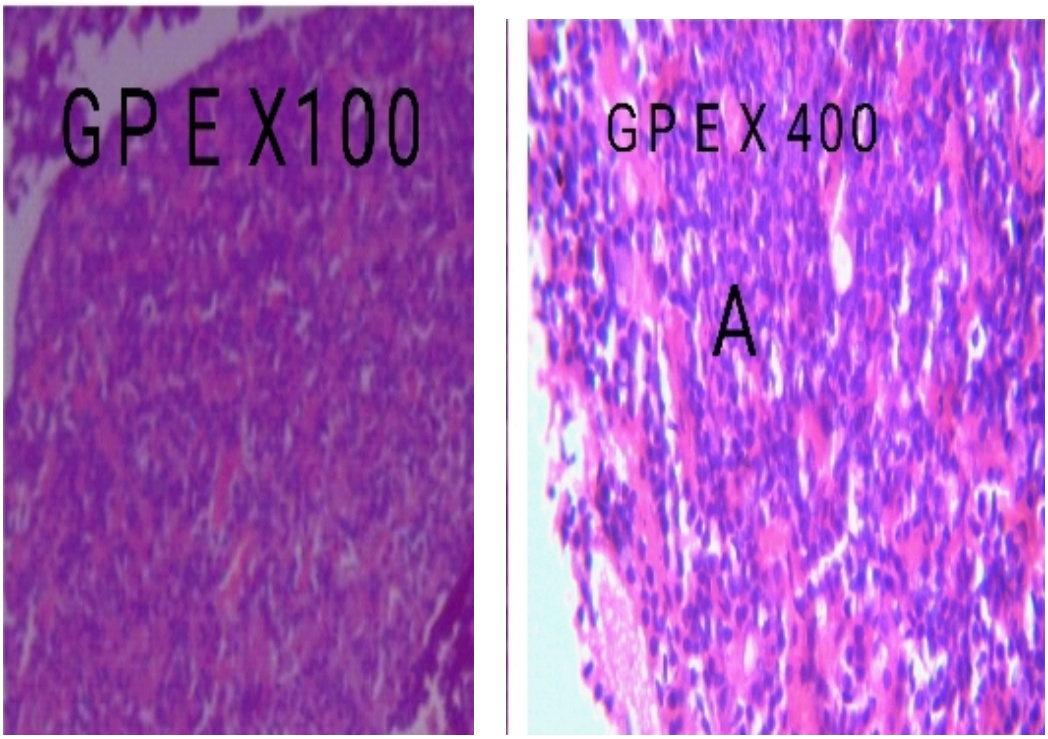

Plate 5: Photomicrograph (X100/X400) (H/E) of section of group E of pituitary gland induced with Aluminum chloride and subsequently treated with 100mg/kg of Vitamin E (Tocopherol) shows increased number of acidophil (A) with active basophil.

4. DISCUSSION

$AlCl_3$ exposure induced oxidative imbalance, as shown by elevated MDA and reduced SOD, CAT, and GSH, consistent with prior findings on aluminum's role in neural and endocrine perturbations (Bondy, 2010; Yokel, 2013; Nampoothiri et al., 2015; Kumar et al., 2009). Hormonal declines underscore pituitary vulnerability to free radicals (Butterfield & Boyd-Kimball, 2005; Verstraeten et al., 2008).

P. domestica extract reversed these effects dose-dependently, attributable to its polyphenolic content (e.g., quercetin, chlorogenic acid) that neutralizes radicals and preserves cellular integrity (Gil et al., 2002; Fraga et al., 2010; Reiter et al., 2001). Histological improvements support this, aligning with studies on antioxidant-mediated tissue protection (Liu et al., 2012; Sharma & Mishra, 2006). Though variability in rat responses (e.g., due to strain or bioavailability) warrants caution, these outcomes echo broader evidence of plant extracts mitigating metal toxicity (Saleem et al., 2010). Limitations include absence of long-term effects and exact extract standardization. Future work could explore human applicability for managing aluminum-related toxicities (Nordberg et al., 2015). Vitamin E's efficacy validates antioxidant-based interventions (Sharma et al., 2007).

5. CONCLUSION

Ethanolic Prunus domestica leaf extract potently alleviates $AlCl_3$-induced oxidative and structural pituitary damage in male Wistar rats via antioxidant and hormonal regulatory pathways, positioning it as a candidate for environmental toxicity mitigation.

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