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Folic acid (Vitamin B9) and cobalamin (Vitamin B12) ameliorate Lead Acetate-induced oxidative stress in the cerebellum of Wistar Rats.

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ABSTRACT
Heavy metals such as lead-Acetate (PbA) poses a major risk, particularly in developing countries, through contaminated water, industrial emissions, and poor waste management. The cerebellum, crucial for balance and coordination, undergoes oxidative stress and neuronal damage from Pb exposure. This study examined whether folic acid (vitamin B9) and vitamin B12 supplements could counteract lead-induced cerebellar damage in adult Wistar rats. Thirty rats were divided into five groups (n=6): Control, PbA (120 mg/kg), FA + VB12 (100 mg/kg), PbA (120 mg/kg) + FA + VB12 (100 mg/kg), and PbA (120 mg/kg) + FA + VB12 (400 mg/kg). Treatments were administered orally for 14 days. Some brain tissue homogenate was used for biochemical studies, while some was used for histological studies. The results showed a significant ($p< 0.05$) rise in lead (PbA) concentration and Malondialdehyde in the PbA-treated group when compared to the control. A significant ($p< 0.05$) decline was observed in FA + VB12 (100 mg/kg) and Pb (120 mg/kg) + FA + VB12 (400 mg/kg) treated groups. Superoxide dismutase, Catalase, and Glutathione Peroxidase activity decreased significantly ($p< 0.05$) in the Pb-treated group when compared to the control, while a significant ($p< 0.05$) increase was observed in Pb (120 mg/kg) + FA + VB12 (400 mg/kg). Histologically, PbA caused cerebellar neuronal shrinkage, and gliosis. FA + VB12 treatment attenuated these changes, preserving neuronal structure. In conclusion, folic acid and vitamin B12 synergistically relieve Pb-induced cerebellar oxidative stress, restore antioxidant defenses, maintain histological integrity, and support motor function, highlighting their therapeutic potential.

Keywords:
Lead-Acetate,
Folic Acid,
Vitamin B12,
Oxidative stress,
Toxicity

INTRODUCTION

Lead toxicity remains a key global public health concern due to its assiduous in the environment and profound adverse health effects, particularly neurotoxicity. The introduction of lead occurs through contaminated air, water, soil, food, and industrial emissions, posing significant risks to developing and adult populations (Sanders *et al.*, 2009; Kumar *et al.*, 2020; Ortega *et al.*, 2021).

Lead is a ubiquitous environmental neurotoxin that elicits widespread organ damage, with the brain being particularly vulnerable to its damaging effects (da Silva *et a1.*, 2020). The mechanisms by which lead employs its toxic effects are multidimensional. The presence of lead interrupts calcium homeostasis (Figure 1 and 2), imitating calcium ions and meddling with numerous calcium-dependent cellular processes (da Silva *et a1.*, 2020).

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It also harms the function of the mitochondrion, which is essential for the manufacturing of cellular energy, leading to the production of ROS and subsequent oxidative impairment. Furthermore, lead affects neurotransmission, disturbing the subtle balance of excitatory and inhibitory signals in the brain (Gudadhe *et al.*, 2024; Tamagno and Freeman, 2025; Krupanshi, 2025). These oxidative mechanisms, coupled with calcium dysregulation and mitochondrial dysfunction, climax in neuronal death and synaptic dysfunction (da Silva et a1., 2020; Jurcău *et al.*, 2022). It is worthy to note that studies have shown that lead exposure substantially decreases SOD and CAT activity in brain tissue and rises markers of lipid peroxidation, such as malondialdehyde (MDA) (Dewanjee *et al.*, 2013; Ferreira *et al.*, 2016; Fan *et al.*, 2020). Lead binds to the -SH groups of GSH, SOD, and CAT, leading to their depletion (Figure 2) and consequently reducing the cell's capacity to neutralize free radicals (Singh *et al.*, 2018; Kennedy *et al.*, 2020; Singh *et al.*, 2023; Vukelić *et al.*, 2023). The reduction in their activity leads to an uncontrolled accumulation of ROS (Singh *et al.*, 2018).

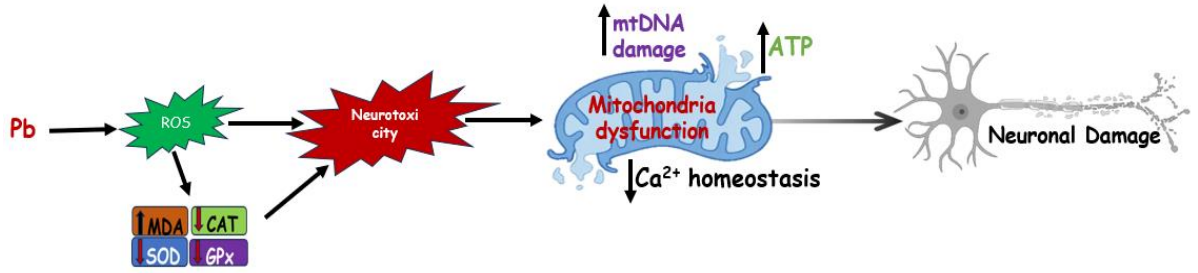

Figure 1: Pathophysiology of lead-induced neurotoxicity within the brain (Auza, 2026). Designed using power point.

This sustained ROS accumulation intensifies oxidative stress, potentially leading to chronic oxidative impairment, and further elevates MDA levels (Kelainy *et al.*, 2019). Reactive oxygen species and LPO can damage the Na^+/K^+-ATPase pump, a key enzyme responsible for preserving the electrochemical gradients across neuronal membranes. Impairment of this Na^+/K^+-ATPase pump results in neuronal depolarization and interference of ion homeostasis, contributing to cellular dysfunction (Singh *et al.*, 2024; Mahapatra *et al.*, 2024; Sidhu *et al.*, 2025).

Folic acid, has been revealed to protect against lead-induced spleen injury and apoptosis, mechanisms driven by oxidative stress, and can lower blood lead levels in lead-poisoned juvenile rats (Abdulwaliyu *et al.*, 2021; Li *et al.*, 2021b).

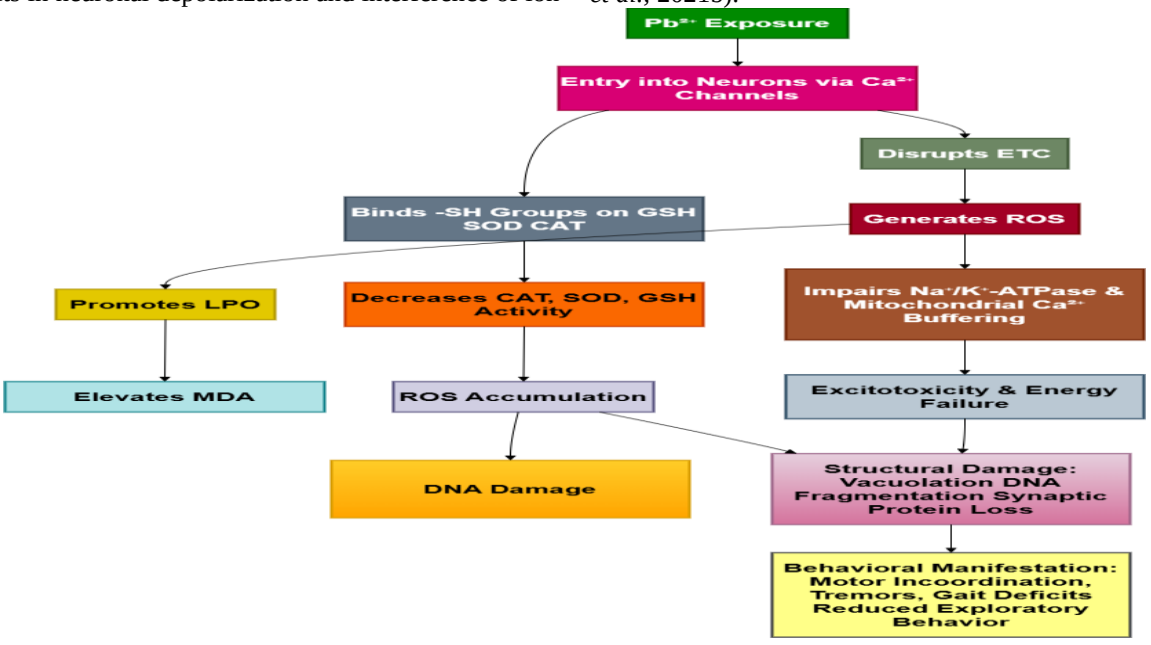

Figure 2: Mechanisms of Lead-Induced Neurotoxicity. (Auza, 2026). Designed using draw.io

Vitamins B9 and B12 are water-soluble B-complex vitamins crucial for one-carbon metabolism, DNA synthesis, and methylation processes that maintain neural health (Han *et al.*, 2025). Folic acid participates in reducing homocysteine levels, an excitotoxic amino acid that worsens oxidative stress and neuronal injury (Ekundayo *et al.*, 2025). Cobalamin acts as a cofactor in enzymatic reactions that promote myelin synthesis and neurological repair, alongside scavenging free radicals directly (Chan *et al.*, 2018). By targeting cerebellar oxidative stress, folic acid and cobalamin improve motor coordination and cognitive processes adversely affected by lead toxicity (Ekundayo *et al.*, 2025). The enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) play a critical role in neutralizing reactive oxygen species (ROS), thus mitigating oxidative stress-induced damage (Ojeaburu & Asiriuwa, 2025). The mitigation effect through antioxidant enhancement suggests a vital neuroprotective strategy that may complement broader public health interventions to limit lead exposure and its neurological sequelae (Moosavirad *et al.*, 2016; Ekundayo *et al.*, 2025). They help maintain redox homeostasis, protect neuronal structures from oxidative damage, and sustain neurophysiological functions by modulating cellular signaling pathways involved in apoptosis (Ekundayo *et al.*, 2025).

MATERIALS AND METHODS

Lead acetate (PbA) (CAS No: 027595) manufacture by Loba Chemie Pvt. LTD, Lagos was obtained the department of pharmaceutical sciences, Bingham University, Karu and Folic acid and Vitamin B12 tablets (Batch No: 15053/29) manufactured by Vitabiotics (NIG) Ltd, was purchased from De Eminent Pharmacy, Kaduna state, Nigeria. Other materials used includes: Cages, water bottles, saw dust for bedding, Animal feed, Digital Weighing balance, Microscope, Amscope, glass slides, cover slips, stains, fixative, bottles for fixing tissues, Distilled water, Beakers, syringes, Dissecting set, Diagnostic Kits, Ketamine, Teflon glass homogenizer, Centrifuge, Phosphate buffer. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and law for laboratory experimentation and received approval from the Research and Ethical Committee, College of Health Science, Bingham University Karu (approval no. BHUCAUC/CHS/2025/005).

Experimental Protocol

In this study, a total of 30 adult male Wistar rats (100-120g) were procured from the animal care unit (ACU), Ahmadu Bello University, Zaria and were randomly divided into 5 groups of 6 rats. The animals were kept and maintained in standard laboratory conditions of room temperature, humidity and under 12-hour dark-light cycle in polyester cage with wire gauze. The control group received distilled water only all through the period of the experiment serving as the positive control while administration of the other the treatment groups were done as follow; PbA (120 mg/kg) received 120 mg/kg lead acetate (20% LD_{50}) (Sujatha *et al.*, 2011). Groups PbA (120 mg/kg), PbA (120mg) + FA and VB12 (100mg/kg), and PbA (120mg) + FA and VB12 (400mg/kg) received 120 mg/kg lead acetate for seven (7) days (days 1 - 7). Groups FA and VB12 only (100mg/kg) received 100 mg/kg of FA and VB12 only all through the 14 days period of administration. Groups PbA (120mg) + FA and VB12 (100mg/kg), and PbA (120mg) + FA and VB12 (400mg/kg) received (in addition to the 120 mg/kg lead acetate), 100 mg/kg and 400 mg/kg of *FA and VB12*, respectively. All administrations were done orally and lasted for 14 days.

Biochemical studies

Each homogenized brain tissue was centrifuged and aliquots of the supernatant were obtained for biochemical/neurochemical analysis. Lipid peroxide levels, Malondialdehyde (MDA) and antioxidant enzymatic activity: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH) and levels of some brain neurotransmitters (dopamine and glutamate) was determined using brain homogenate. The concentration of Malondialdehyde (MDA) as an index of lipid peroxidation was evaluated in the samples using an assay kit (Rat Malondialdehyde (MDA) ELISA Kit; WKEA Med Supplies Corp, China) as stated by the manufacturer. The principle was based on the method reported by Okey and Ayo (2015). Superoxide dismutase activity was analyzed using Rat Super Oxidase Dismutase (SOD) ELISA Kit (WKEA Med Supplies Corp, China) as stated by the manufacturer. The procedure was based on the method reported by Okey and Ayo (2015). Catalase activity was determined using Rat Catalase (CAT) ELISA Kit (WKEA Med Supplies Corp, China) according to the manufacturer's instructions. The procedure used was based on the method reported by Okey and Ayo (2015). Reduced Glutathione was analyzed using Glutathione Peroxidase (GSH) ELISA Kit (WKEA Med Supplies Corp, China) as stated by the manufacturer. The procedure was based on the method of (Ursini *et al.*, 1995).

Data Analysis

Data obtained were analyzed using GraphPad Prism (version, 9.5.1), results obtained were expressed as mean ± S.E.M, comparison between groups was determined using one-way Analysis of Variance (ANOVA) and the presence of significant differences among means was determined using *Tukey honestly significant difference* (*HSD*) as *post hoc* test for significance. Values were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Biochemical

A significant (p<0.05) increased level of lead (Pb) concentration was observed in the brain tissue of Wistar rats in the lead acetate group compared to the control, and a significant (p<0.01) decrease was observed in the treatment PbA + VB12 (100 mg/kg and 400 mg/kg) groups when compared to group PbA (120 mg/kg) figure 3.

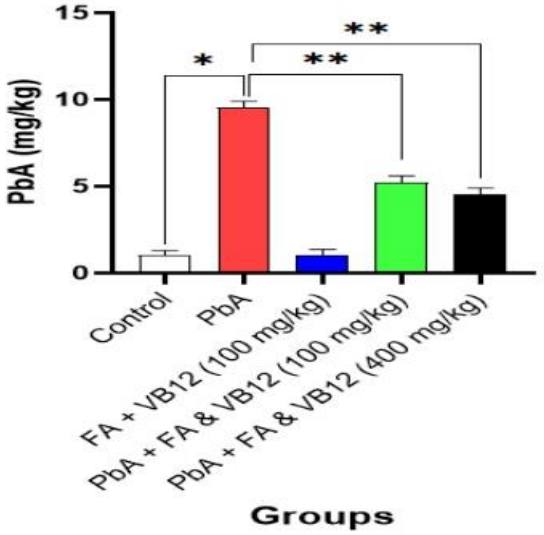

Figure 3: Effect of Folic acid and VB12 on Lead (Pb) concentration in Wistar rats

n = 6, mean ± SEM, One-way ANOVA, *=p<0.05, **=p<0.01, ***=p<0.001when compared to the PbA group. **PbA** = Lead acetate (120 mg/kg), **FA & VB12** = Folic acid and Vitamin B12 (100 mg/kg; and 400 mg/kg).

Biochemistry of Lipid Peroxidation and Antioxidant Enzyme Activity

Tissue MDA levels increased significantly in the Pb-treated group when compared to the control, an indication of lipid peroxidation. A significantly (p<0.05) lower level was observed FA + VB12-treated (100 mg/kg) and PbA + FA & VB12 (100 mg/kg and 400 mg/kg) groups when compared to the PbA-treated group **(Figure 4A).** Tissue CAT activity levels in PbA + FA & VB12 (100 mg/kg and 400 mg/kg) groups showed a remarkable (p<0.05) increase compared to the PbA only group, while a significantly (p<0.05) decreased CAT activity level was observed in PbA-treated group **(Figure 4B).** SOD activity decreased significantly in the PbA-treated group when compared to the control **(Figure 4C).** Tissue GPx levels decreased significantly (p<0.05) in the PbA-treated group when compared to the control when compared to the control while a significant (p<0.05) increase was observed in the PbA + FA & VB12 (400 mg/kg)-treatment group (**Figure 5D).**

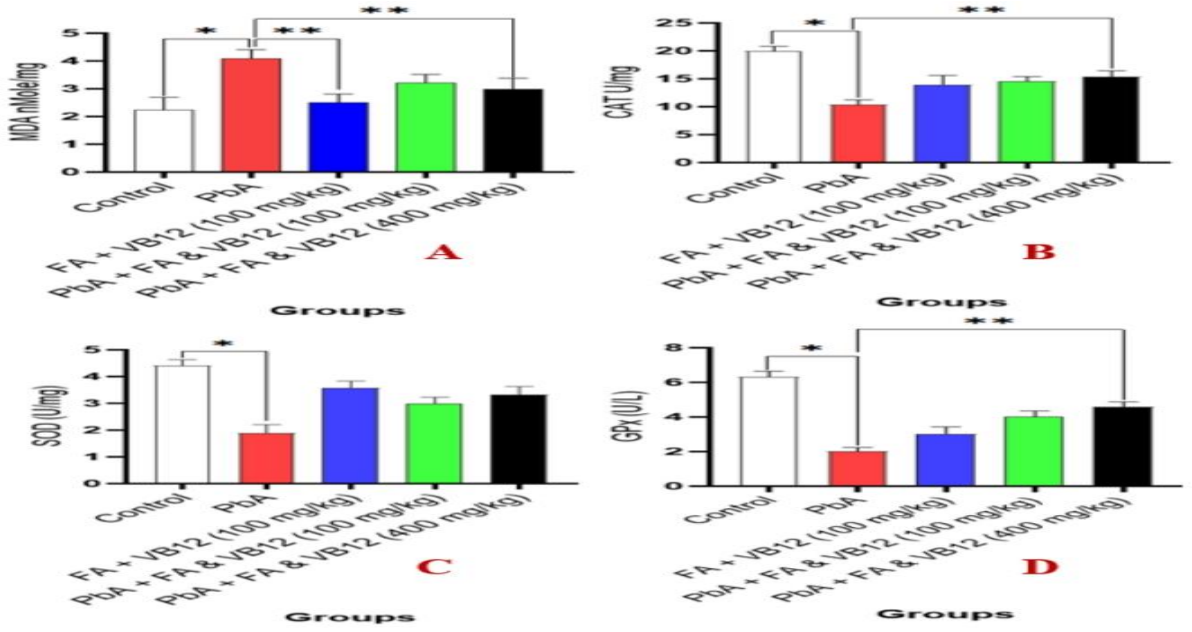

Figure 4: Effect of Folic acid and VB12 on Antioxidant Enzyme Activity in Wistar rats

n = 6, mean ± SEM, One-way ANOVA, *=p<0.05, **=p<0.01, ***=p<0.001when compared to the PbA group. **PbA =** Lead acetate (120 mg/kg), **FA & VB12 =** Folic acid and Vitamin B12 (100 mg/kg; and 400 mg/kg).

Histology

Rats in the control and FA & VB12 groups had a normal panoramic morphological presentation of the cerebellum at very various exposure and magnification (Figure 5). The well-outlined array of Purkinje cells was distinctly arranged. PbA treatment, on the other hand, showed degenerative changes in the cerebellum and was characterized by neuronal cytoplasmic shrinkage of Purkinje cells. However, Examination of the cerebellar sections of Wistar rats treated with FA & VB12, revealed mild distorted cytoarchitecture of the cerebellar cortex with relatively preserved histological features when compared to the control (Figure 5).

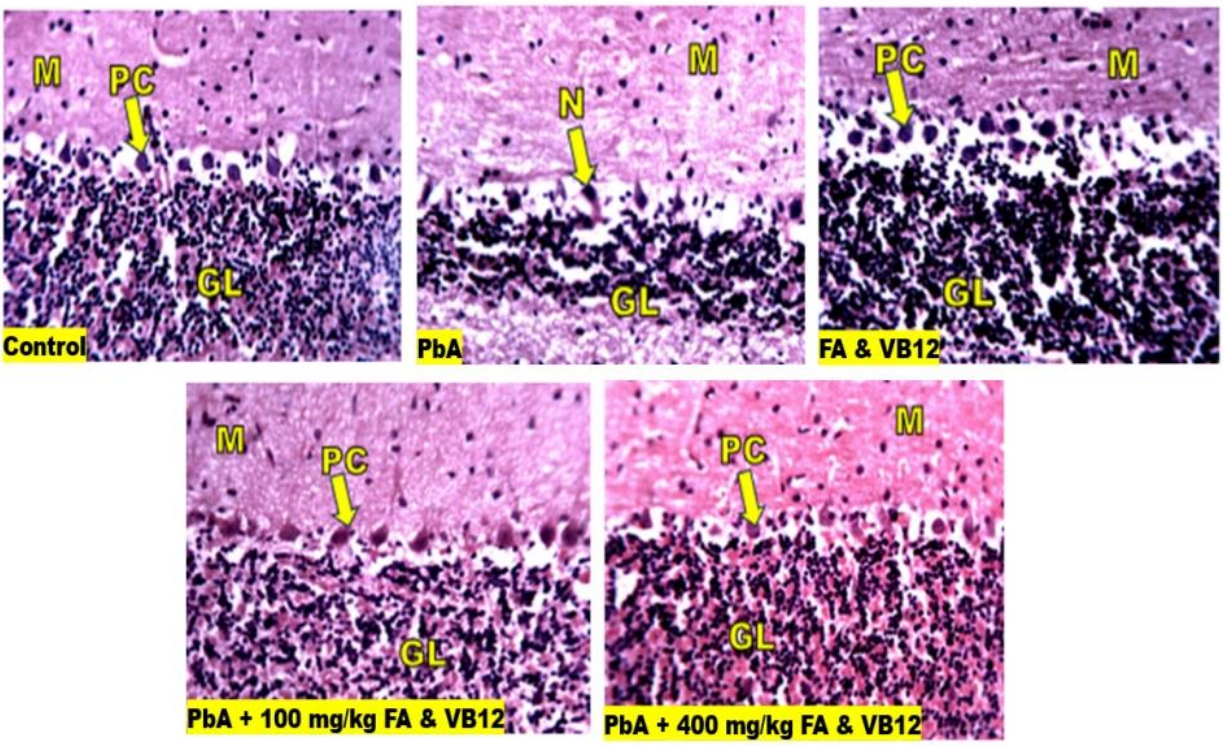

Figure 5: Photomicrographs of the cerebellum showing the cytoarchitecture. PC: Purkinje cell; G: Granular layer; M: Molecular layer. (Mag x 250 H&E). **Control** showing normal cytoarchitecture. PC: Purkinje cell; G: Granular layer; M: Molecular layer. **PbA** showing loss of Purkinje cells, Neuronal degeneration (N) characterized by neuronal cytoplasmic shrinkage of Purkinje cells. **FA & VB12** showing preserved cytoarchitecture. P: Purkinje cell; G: Granular layer M: Molecular layer. **PbA + 100 mg/kg FA & VB12** showing mild distortion in the cytoarchitecture. P: Purkinje cell; G: Granular layer M: Molecular layer. **PbA + 400 mg/kg FA & VB12** showing mild distortion in the cytoarchitecture. P: Purkinje cell; G: Granular layer M: Molecular layer.

Exposure to lead acetate elevates lead concentrations in the brain tissue of Wistar rats significantly, comparing with a marked increase in oxidative stress and a simultaneous reduction in antioxidant enzyme activities. This complex interplay of toxicity and oxidative imbalance is a well-recognised phenomenon in lead poisoning, and recent findings further highlight the protecting roles of folic acid (FA) and vitamin B12 (VB12) supplements in mitigating these adverse effects (Abd allah and Badary, 2017; Enogieru and Iyoha, 2023). The lead acetate (PbA) group revealed a substantial elevation in brain lead concentration compared to the control group, signifying successful induction of lead neurotoxicity in the experimental model. This finding is consistent with numerous studies stressing lead's tendency to accrue in different tissues, including the brain, where it exerts neurotoxic effects (da Silva *et al.*, 2020). The PbA group demonstrated a Pb concentration of approximately 9.2 mg/kg in wet cortical tissue, significantly higher than the control and FA +VB12-only groups. Notably, the groups treated with PbA + VB12 (100 mg/kg and 400 mg/kg) showed a significant reduction in brain lead levels compared to the PbA group, suggesting that VB12 supplementation helps to reduce lead build-up in brain tissue. This aligns with previous

research indicating that certain interventions can reduce lead incumbrance in biological systems (Dede *et al.,* 2018).

Oxidative stress, portrayed by an imbalance between pro-oxidant and antioxidant systems, is a primary mechanism underlying lead toxicity (Dewanjee *et al.,* 2013; Ferreira *et al.,* 2017). Higher malondialdehyde levels were observed in the PbA group than in controls, this points straight to more lipid breakdown, a hallmark of oxidative damage to cellular membranes. Noticeable increase in MDA values appears clearly when viewing PbA against the control. Since earlier studies show lead triggers oxidative imbalance in different tissues (Dewanjee *et al.,* 2013; Amin *et al.,* 2020). Importantly, the groups treated with FA + VB12 (100 mg/kg) and PbA + FA & VB12 (100 mg/kg and 400 mg/kg) unveiled significantly lower MDA levels compared to the PbA-treated group. This implies that the combined supplementation of folic acid and vitamin B12 effectively lessens lead-induced lipid peroxidation, supporting their antioxidant properties (Abd allah & Badary, 2017; Banerjee *et al.,* 2019; Li *et al.,* 2021).

Notably, exposure to lead weakened the body's natural defenses against oxidative stress. The group exposed to lead acetate, antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase - dropped sharply when measured next to untreated subjects. While catalase activity declined in the PbA group lead, those given PbA + FA & VB12 (100 mg/kg and 400 mg/kg) saw a clear rebound in enzyme performance, hinting at recovery supported by these nutrients. A similar pattern appeared with superoxide dismutase: significantly reduced in the PbA group, but stabilized with intervention. Glutathione peroxidase followed suit, showing diminished presence following toxic insult. Such drops suggest cells struggle to shield themselves once lead disrupts normal redox balance (da Silva *et al.*, 2020).

Though exposed to lead, animals receiving folic acid and vitamin B12 showed stronger defense mechanisms. Notably, those treated with 400 mg/kg of these nutrients had higher GPx levels than control group. The findings suggest these FA and VB12 support the cell's ability to counteract harmful oxidation, thereby maintaining and restoring cellular antioxidant capacity, which is crucial for mitigating lead-induced oxidative damage. Protection emerges more clearly when folic acid and vitamin B12 supply remains consistent under toxic stress. These supplements reduce lead levels, thereby decreasing lipid peroxidation, and enhancing antioxidant enzyme activities. This aligns with the research objective. This consistency underscores their potential as therapeutic or preventive agents in managing lead toxicity. Lead exposure interferes with key biological functions, including DNA synthesis and methylation, processes where folic acid is essential (Abd allah & Badary, 2017; Li et al., 2021).

Vitamin B12 is essential for methionine synthase activity, which is crucial for recycling homocysteine and regenerating active folate, thus preventing the "methyl-folate trap" that can lead to functional folate deficiency and impaired DNA synthesis. Lead's impact on these pathways likely contributes to the observed oxidative stress and cellular damage. Therefore, supplementation with these vitamins can help maintain cellular integrity and function under lead exposure by supporting these critical metabolic processes and enhancing antioxidant defenses (Abd allah & Badary, 2017; Banerjee *et al.,* 2019). Vitamin B12 keeps methionine synthase working properly, a key step needed so homocysteine gets recycled while active folate forms again, this prevents “methyl-folate trap” that can lead to functional folate deficiency and blocks proper DNA production. When lead interferes on this pathway, it probably worsens oxidative stress and contributes to cellular damage. Taking supplementation of these vitamins can help maintain cellular integrity and function under lead exposure by supporting these critical metabolic processes and enhancing antioxidant defenses (Abd allah & Badary, 2017; Banerjee *et al.,* 2019).

Findings back up what we already know about lead’s harmful effects on the nervous system and its ability to boost oxidation inside cells. At the same time, they add weight to growing proof that folic acid along with vitamin B12 may shield tissues from such harm. Past studies have pointed in this direction too - nutrients acting like shields when metals enter the body (Bislimi *et al.,* 2021; Kahalerras *et al.,* 2022; Enogieru and Iyoha, 2023). Brain tissue showed less lead after giving vitamin B12, thereby revealing that vitamin B12 might alter the pharmacokinetics or distribution of lead. The reversal of elevated MDA and restoration of CAT, SOD, and GPx activities by FA and VB12 demonstrate their direct involvement in bolstering the antioxidant defense system, thus protecting against lead-induced oxidative damage. Though results point to real-world health benefits, the value lies in how they match earlier work on folic acid and B12. Protection seen again in Wistar rats hints at a way to soften harm caused by lead, particularly where risk runs high (Tran *et al.,* 2010; Andjelkovic *et al.,* 2019). Turning lab outcomes into medical guidance demands more study, yet support from past data strengthens confidence in these nutrients’ role against toxic metals. When certain biological markers shift under treatment, clues emerge - not just about how lead damages cells, but how vitamins may step in to shield them.

Histologically, among the observed tissue-level changes, damage caused by lead acetate stands out through shrinking cell bodies and a decrease in the number of Purkinje neurons in the cerebellum of the Wistar rats. These structural alterations and patterns echo earlier

reports linking lead exposure to nerve cell breakdown, especially where metabolic strain disrupts signaling pathways (Fu-shi *et al.,* 2012; Li *et al.,* 2021b). Instead of worsening steadily, some sections showed stability when folic acid combined with vitamin B12, hinting their presence may shield vulnerable structures. Rather than acting alone, these vitamins appear stronger together, softening harm triggered by toxic metal buildup. Looking at the control group, the cerebellum showed typical organization, Purkinje cells were clearly visible, alongside a neatly arranged granular layer and an intact molecular layer. Such a structure provides a reference point, confirming what a healthy cerebellum looks like without lead influence. In contrast, animals exposed to lead revealed clear signs of neural damage: fewer Purkinje cells appeared, while many neurons looked shrunken due to condensed cytoplasm. These changes match earlier findings showing how lead enters the brain by crossing protective barriers and settles in areas such as the cerebellum, triggering forms of programmed and accidental cell death (Fu-shi *et al.,* 2012). Instead of functioning normally, mitochondria under lead stress produce excess reactive molecules, suffer energy deficits, and disturb chemical signaling between nerve cells - a pattern tied directly to deterioration seen in brain tissue (Banerjee *et al.,* 2019; Li *et al.,* 2021b). Among those given folic acid and cobalamin, both groups secured from lead (Folic Acid & VB12-treated) (without PbA), and the one exposed to lead but treated (PbA + FA & VB12 groups) revealed obvious improvement in brain tissue damage caused by lead. Protection of cell structure was evident in the group receiving the supplements without lead (FA & VB12-treated), mirroring healthy controls, reiterating the non-toxic nature and possible neurotrophic effects of these vitamins, and pointing to safety and possible support for nerve cell health. Even with lead exposure, treatment groups displayed far less disruption. Animals receiving either 100 or 400 mg/kg of the supplement combo maintained relatively intact layers of the cerebellum, including Purkinje neurons and the surrounding granular and molecular layers. This indicates that FA and VB12 supplementation effectively mitigated the severe neuronal damage observed in the PbA-only group. Though higher dosages brought greater resilience, even minimal dosing (100 mg/kg) offered significant improvement, implying a robust protective capacity.

This protective role fits how folic acid and vitamin B12 function inside cells. Inside the body, folic acid supports one-carbon transfer reactions - needed for building and fixing DNA, along with chemical tagging such as adding methyl groups to myelin basic protein, a key component for healthy nerve cells (Abd allah and Badary, 2017). When lead enters the system, it blocks those same pathways, resulting in faulty DNA maintenance, higher amounts of homocysteine, and cellular harm caused by unstable molecules (Li *et al.,* 2021b). Acting behind the scenes, vitamin B12 helps activate methionine synthase - an enzyme that turns homocysteine into methionine while restoring tetrahydrofolate, a form required for proper folic acid activity (Banerjee *et al.,* 2019). Together, their presence may offset some of lead's toxic interference, helping preserve brain cell structure and slow deterioration.

These results shape our grasp of lead-related brain damage and possible ways to respond. What stands out is how the structure inside the cerebellum stays intact, especially those Purkinje cells tied to motor coordination and cognitive functions via the neuroprotective capacity of folic acid and vitamin B12, shielding them. In line with earlier work, folic acid appears capable of quieting down inflammation and lipid peroxidation in cells harmed by lead (Abd allah and Badary, 2017). Beyond that, evidence points to it guarding spleen tissue in animals exposed to lead, blocking cell death sparked by internal stress caused by oxidation and overloaded cellular systems (Li et al., 2021b). Even more telling, young rats poisoned by lead show lower amounts of the metal in their blood when given folic acid (Fu-shi *et al.,* 2012).

CONCLUSION

Overall, findings indicate folic acid combined with vitamin B12 protects the cerebellum in Wistar rats against damage from lead acetate. When administered at once, these nutrients sharply reduce neuron loss - highlighting their joint protective role. Despite differences in response, outcomes consistently point toward improved neural resilience under dual treatment. Structural details in the brain tissue stay more intact under combined treatment. Protection likely comes from how these vitamins manage cell oxidation and support metabolism. Even though outcomes point toward strong defense in neural tissues exposed to toxic metals, unknowns remain about lasting impacts over time. A deeper investigation must clarify exactly how this protection works at a molecular level.

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Conflicts of Interest:

The authors report no conflicts of interest in relation to this work.

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