Protective Effects of Andrographis paniculata aqueous leaf extract against lead induced hematological toxicity in male Wistar rats

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ABSTRACT

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| **Aims**: This study aimed to evaluate the protective effects of *Andrographis paniculata* (AP) aqueous leaf extract against lead-induced hematological toxicity in male Wistar rats.  **Study Design:** A randomized controlled animal study.  **Place and Duration of Study:** Department of Biochemistry, University of Port Harcourt, Nigeria, conducted over 28 days.  **Methodology:** Thirty male Wistar rats (90–120 g) were randomly assigned into five groups (n=6). Group I (control) received no treatment, Group II was administered lead acetate (40 mg/kg), while Groups III, IV, and V were co-treated with AP at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg, respectively, alongside 40 mg/kg lead acetate. Following 28 days of treatment, blood samples were collected to assess blood lead levels and hematological indices, including hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV). Data were analyzed using one-way ANOVA, with statistical significance set at P < 0.05.  **Results**: Lead exposure significantly reduced Hb (8.50 ± 0.40 g/dL), RBC (4.20 ± 0.10 ×10⁶/µL), and packed cell volume (PCV: 24.00 ± 1.00%), while increasing WBC (16.83 ± 0.35 ×10³/µL) and PLT (615.33 ± 237.79 ×10³/µL) compared to controls. AP treatment dose-dependently mitigated these effects, with Group V (300 mg/kg AP) restoring Hb (13.30 ± 0.53 g/dL), RBC (6.80 ± 0.10 ×10⁶/µL), and PCV (38.67 ± 2.08%) to near-normal levels. A significant reduction in blood lead levels was also observed in AP-treated groups, particularly at 300 mg/kg.  **Conclusion:** *Andrographis paniculata* aqueous leaf extract exhibits protective effects against lead-induced hematological toxicity, likely through its antioxidant and anti-inflammatory properties. These findings support its potential as a natural therapeutic agent for mitigating lead toxicity. Further studies are warranted to elucidate its precise mechanisms of action. |

***Keywords:*** *Anemia, Andrographis paniculata, Wistar Rat, hematotoxicity*

1. INTRODUCTION

Lead has been utilized by humans for thousands of years due to its desirable properties, such as a low melting point, corrosion resistance, and high density. However, exposure to lead occurs primarily through inhalation and ingestion, leading to increased blood lead levels following intestinal absorption (Ray, 2016). Lead toxicity is associated with numerous adverse effects, including neurological, hematological, reproductive, renal, cardiovascular, and gastrointestinal complications (Debnath et al., 2019). The toxic effects of lead are particularly severe in children due to their increased vulnerability, as their developing tissues are more susceptible to damage. Even at low exposure levels, lead has been linked to behavioral problems, learning deficits, and reduced IQ in children (Rubin & Strayer, 2008).

Among the various biological systems affected by lead exposure, the hematopoietic system is particularly vulnerable. Hemoglobin and erythrocytes are among the most affected components, with approximately 99% of absorbed lead binding to erythrocytes, where 85% is bound to heme (Ray, 2016). Lead interferes with hemoglobin synthesis by inhibiting key enzymes in the heme biosynthesis pathway, including δ-aminolevulinic acid dehydratase (ALAD), aminolevulinic acid synthetase (ALAS), and ferrochelatase (Dongre et al., 2011). These disruptions result in microcytic anemia, characterized by small red blood cells with compromised oxygen-carrying capacity. Additionally, lead exposure increases erythrocyte membrane fragility, leading to hemolysis and a reduced lifespan of circulating red blood cells (Kianoush & Balali, 2013). One of the earliest hematological markers of lead poisoning is basophilic stippling of erythrocytes, which results from the accumulation of ribonucleic acid degradation products (Ray, 2016). Chronic lead exposure is also associated with increased blood pressure, particularly in middle-aged and older individuals (Bergdahl & Skerfving, 2008).

Beyond its hematological effects, lead induces oxidative stress by inhibiting oxidative enzymes, leading to excessive production of reactive oxygen species (ROS) and subsequent cellular damage (Wani et al., 2015). Hemoglobin oxidation triggered by lead exposure promotes red blood cell hemolysis, further exacerbating anemia (Laksana et al., 2022). Additionally, lead disrupts vascular integrity by altering collagen synthesis and increasing blood vessel permeability, ultimately impairing immune function (Kosnett, 2006). Lead-induced anemia results from impaired heme synthesis, with ALAD inhibition preventing the conversion of aminolevulinic acid (ALA) into porphobilinogen, a key step in heme biosynthesis (Patrick, 2006). This disruption leads to the accumulation of heme precursors, which have been implicated in neuronal toxicity (Dongre et al., 2011).

Andrographis paniculata (Family: Acanthaceae) is a medicinal plant widely distributed across subtropical regions, including India, China, Vietnam, Malaysia, Thailand, and Nigeria. Due to its intensely bitter taste, A. paniculata is commonly referred to as the "King of Bitters" and has been traditionally used as a tonic for various ailments. Phytochemical analyses of AP have identified bioactive compounds such as andrographolide, deoxyandrographolide, quercetin, kaempferol, and apigenin, which contribute to its therapeutic properties (Rammohan et al., 2008). A recent study by Chau et al. (2024) identified 3-O-β-d-glucosyl-14-deoxyandrographiside and 14-deoxyandrographolide as key bioactive compounds in the plant. Research shows that the plant has antioxidant, anti-inflammatory, antibacterial, anticancer, and hepatoprotective properties (Mehta et al., 2021). Additionally, andrographolide has been reported to inhibit platelet aggregation, which suggests a potential role in preventing thrombotic disorders and improving blood circulation (Owoade et al., 2021).

Given its extensive pharmacological properties, AP may offer a promising natural alternative for mitigating lead-induced hematological toxicity. Studies have demonstrated its potential in supporting immune function, reducing inflammation, and protecting against oxidative damage (Alasyam et al., 2017). This study aims to evaluate the protective effects of AP aqueous leaf extract on hematological indices in lead-exposed Wistar rats, providing insights into its potential therapeutic applications.

2. material and methods

**2.1 Reagents, Chemicals, and Diets**

The reagents and chemicals used in this study were of analytical grade and included chloroform (BDH Chemicals Ltd.), ethanol (95%), formalin (10%), biochemical reagent kits (Randox), and Blue Crown 9mm feed (Top Feed Ltd.). Lead acetate was obtained from Sigma-Aldrich.

**2.2 Plant Material**

Fresh leaves of Andrographis paniculata were collected from a farmland at Choba, Delta Campus, University of Port Harcourt, Rivers State, Nigeria. The leaves were identified and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt, where a voucher specimen was deposited in the departmental herbarium.

**2.3 Preparation of Plant Extract**

The collected leaves were cleaned and air-dried at room temperature (30 ± 2°C) for seven days before being pulverized into fine powder using a laboratory mechanical grinder. The powdered leaves were stored in airtight Tarson bottles at 20°C until further use. To prepare the aqueous extract, 50 g of the powdered leaves were soaked in 600 mL of distilled water overnight. The mixture was filtered through muslin cloth and centrifuged at 5000 rpm for 10 minutes. The supernatant was filtered again, collected in sterile polypropylene tubes, and frozen at -20°C before being lyophilized for further use.

**2.4 Animal Handling and Experimental Design**

Thirty (30) adult male Wistar rats (90–120 g) were housed in the University of Port Harcourt animal house under standard laboratory conditions of temperature, humidity, and unrestricted access to pelletized rat chow and water. The animals were allowed to acclimatize for one week before the study commenced.

The rats were randomly assigned into five groups (n = 6 per group) as follows:

Group I (Control): Received no treatment.

Group II (Lead-Treated): Administered 40 mg/kg lead acetate.

Group III: Administered 40 mg/kg lead acetate + 100 mg/kg Andrographis paniculata extract.

Group IV: Administered 40 mg/kg lead acetate + 200 mg/kg Andrographis paniculata extract.

Group V: Administered 40 mg/kg lead acetate + 300 mg/kg Andrographis paniculata extract.

The lead acetate was administered orally using an oral gavage, 20 minutes after the administration of AP extract. Treatment was given once daily for 28 consecutive days. The dosage of lead acetate (40 mg/kg) was selected based on its established toxicity profile, where an LD50 of 150 mg/kg body weight has been reported (Debosree et al., 2012). The A. paniculata dosage was determined based on previous studies that calculated its LD50 to be greater than 3000 mg/kg body weight (Nasir et al., 2013).

The body weight of the animals was recorded weekly to monitor any changes during the experiment.

**2.5 Sample Collection**

At the end of the 28-day treatment period, the rats were sedated using diethyl ether-soaked cotton wool in a desiccator. Blood samples were collected via the jugular vein into ethylenediaminetetraacetic acid (EDTA) tubes for blood lead level (BLL) determination and hematological analysis.

**2.6 Determination of Blood Lead Levels**

Blood samples were digested using a mixture of concentrated nitric acid and sulfuric acid, followed by oxidation with hydrogen peroxide. The pH of the digested samples was adjusted to approximately 3.0 using concentrated ammonia before lead was extracted into isobutyl methyl ketone using ammonium pyrrolidine dithiocarbamate (APDC). Lead concentration was then quantified using Atomic Absorption Spectroscopy (AAS), with a calibration curve prepared by plotting the absorbance of standard solutions against their known concentrations.

**2.7 Determination of Hematological Indices**

Hematological parameters were analyzed using an auto-hematology analyzer (URIT-2900Vet Plus). This analyzer measures changes in electrical resistance as blood cells pass through a detection aperture, enabling the enumeration of red blood cells (RBC), white blood cells (WBC), and platelet counts, along with their corresponding histograms. The analyzer was properly calibrated before sample analysis, and 13 µL of each blood sample was aspirated for automated measurement. Results for all hematological indices, including hemoglobin (Hb), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), were recorded automatically within 30–60 seconds after sample aspiration.

3. results and discussion

**3.1 Effect of aqueous extract of leaves of *Andrographis paniculata* on the blood lead level of lead acetate induced toxicity in male Wistar rats**

The study revealed a dose-dependent reduction in blood lead levels (BLL) among AP-treated groups (Table 1).

**Table 1: Body weight (g) of rat groups after 4 weeks of treatment**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Rat groups** | **Body weight (g)** | | | |
|  | **Week 1** | **Week 2** | **Week 3** | **Week 4** |
| **Group I** | 100.93 ± 18.96a | 120.87 ± 19.50a | 138.73 ± 22.13a | 166.88 ± 23.57ab |
| **Group II** | 97.22 ± 18.13a | 112.52 ± 13.42a | 132.08 ± 18.46a | 169.08 ± 19.35a |
| **Group III** | 98.08 ± 10.82a | 119.95 ± 6.94 a | 132.51 ± 5.90a | 159.42 ± 5.26ab |
| **Group IV** | 95.13 ± 10.82a | 112.23 ± 11.21a | 124.10 ± 9.44a | 147.50 ± 16.34b |
| **Group V** | 95.78 ± 6.66a | 111.88 ± 5.44a | 123.70 ± 8.91a | 148.62 ± 7.99ab |

Values are means ± standard deviation of 6 rats per group. Values in the same column bearing the same superscript letters are not significantly different at 5% level.

The results (Table 2) showed a reduction in blood lead levels in Group V (0.02 ± 0.02 mg/L) compared to Group II (0.18 ± 0.05 mg/L), indicating that AP extract may facilitate lead excretion. In contrast, Groups III and IV exhibited higher blood lead concentrations compared to the control (0.03 ± 0.04 mg/L), suggesting initial redistribution of lead from tissues into circulation. This phenomenon has been previously observed in chelation therapy, where lead mobilization from bones and soft tissues results in transient increases in blood lead levels before eventual excretion (Assi et al., 2016). The ability of AP to lower blood lead levels may be attributed to its antioxidant properties, which promote detoxification and mitigate oxidative stress induced by lead exposure (Fardiyah et al., 2020).

**Table 2 Effect of aqueous extract of leaves of *Andrographis paniculata* on blood lead level of lead acetate exposed male Wistar rats after 4 weeks.**

|  |  |
| --- | --- |
| Rats groups | Blood lead conc. (mg/l) |
| Group 1 (Control | 0.03± 0.04ᵇ |
| Group 2 (Lead treated 40mg) | 0.18 ± 0.02ᵇᶜ |
| Group 3 (Lead treated 40mg + extract 100mg) | 0.07 ± 0.05ᵃ |
| Group 4 (Lead treated 40mg+ extract 200mg) | 0.07± 0.06ᵃ |
| Group 5 (Lead treated 40mg + extract 300mg) | 0.02± 0.02ᶜ |

Values are means ± standard deviation of triplicate determinations. Values in the same column bearing the same superscript letters are not significantly different at 5% level.

**3.2 Effect of Andrographis paniculata on Hematological Indices**

Lead exposure in Group II induced significant hematological toxicity, characterized by decreased hemoglobin (Hb: 8.50 ± 0.40 g/dL), red blood cells (RBC: 4.20 ± 0.10 ×10⁶/µL), and packed cell volume (PCV: 24.00 ± 1.00%) compared to the control group (Table 3). These findings align with previous studies demonstrating lead’s inhibitory effects on δ-aminolevulinic acid dehydratase (ALAD), a key enzyme in heme biosynthesis (Ray, 2016). The suppression of ALAD leads to impaired hemoglobin production and microcytic anemia, a well-documented consequence of chronic lead toxicity (Patrick, 2006).

**Table 3 Effect of aqueous extract leaves of *Andrographis paniculata* on the hematological indices of lead acetate exposed male Wistar albino rats after 4 weeks treatment**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Hematological indices | Group 1  (Control) | Group 2  (Lead Treated 40mg) | Group 3  (Lead Treated 40mg + Extract 100mg) | Group 4 (Lead Treated 40mg+ Extract 200mg) | Group 5  (Lead Treated 40mg+Extract 300mg) |
| PCV (g/dl) | 34.33 ± 4.04ᵃ | 24.00± 1.00ᵇ | 32.67 ± 6.51ᵃ | 36.00 ± 1.00ᵃ | 38.67 ± 2.08ᵃ |
| HB (%) | 11.70 ± 1.05ᵃᵇ | 8.50 ± 0.40ᶜ | 11.00± 2.30ᵇ | 12.30± 0.30ᵃᵇ | 13.30 ± 0.53ᵃ |
| RBC (cell/μL) | 6.50 ± 0.26ᵃ | 4.20± 0.10ᵇ | 5.77± 1.16ᵃ | 6.57 ± 0.21ᵃ | 6.80 ± 0.10ᵃ |
| WBC (cell/μL) | 4.50 ± 0.30ᶜ | 16.83± 0.35ᵃ | 14.07 ± 6.30ᵃᵇ | 10.10 ± 5.73ᵃᵇᶜ | 8.33 ± 0.87ᵇᶜ |
| PLT (cell/μL) | 184.33 ± 10.69ᵇ | 615.33± 237.79ᵃ | 445.67± 191.01ᵃᵇ | 424.67 ± 179.98ᵃᵇ | 396.67± 178.63ᵃᵇ |
| MCHC (g/dl) | 35.27 ± 1.07ᵃ | 34.73± 1.64ᵃ | 33.43 ± 1.16ᵃ | 33.80 ± 0.36ᵃ | 33.40 ± 0.96ᵃ |
| MCH (pg) | 19.17 ± 0.75ᵃᵇ | 20.20 ± 0.89ᵃ | 19.47 ± 0.25ᵃᵇ | 18.37 ± 0.49ᵇ | 19.57 ± 0.32ᵃ |
| MCV (fL) | 56.03 ± 0.75ᵇᶜ | 58.4*3 ± 0.97*ᵃ | 56.37 ± 0.95ᵃᵇᶜ | 55.57 ± 0.25ᶜ | 58.20 ± 2.09ᵃᵇ |
| N (cell/μL) | 3.33 ± 1.53ᵃ | 6.33 ± 4.04ᵃ | 8.00 ± 4.58ᵃ | 5.33 ± 2.30ᵃ | 7.67 ± 2.08ᵃ |
| L (cell/μL) | 92.33 ± 2.52ᵃ | 88.33 ± 8.02ᵃ | 85.33 ± 4.58ᵃ | 88.67 ± 3.51ᵃ | 85.33 ± 5.51ᵃ |
| E (cell/μL) | 1.33 ± 0.58ᵃ | 2.00 ± 1.73ᵃ | 2.00 ± 1.00ᵃ | 1.67 ± 0.58ᵃ | 2.67 ± 1.53ᵃ |
| M (cell/μL) | 3.00 ± 1.00ᵃ | 3.33 ± 2.52ᵃ | 4.67 ± 0.58ᵃ | 4.33 ± 1.15ᵃ | 4.33 ± 2.08ᵃ |

Values are means ± standard deviation of triplicate determinations. Values in the same row bearing the same superscript letters are not significantly different at 5% level.

**Key: PCV- packed cell volume, HB-hemoglobin, RBC- red blood cell, WBC-white blood cell, PLT-platelet count, MCHC-mean corpuscular hemoglobin concentration, MCH- mean corpuscular hemoglobin, MCV-mean platelet volume, N- neutrophils, L- lymphocytes, E-eosinophils, M- monocyte.**

Administration of APextract resulted in a dose-dependent improvement in hematological indices. Group V (300 mg/kg AP) exhibited significantly restored Hb (13.30 ± 0.53 g/dL), RBC (6.80 ± 0.10 ×10⁶/µL), and PCV (38.67 ± 2.08%), surpassing even control values. These restorative effects can be attributed to the bioactive compounds in AP, such as andrographolide and flavonoids, which possess antioxidant properties that protect erythrocytes from oxidative damage and stabilize the heme synthesis pathway (Tandon et al., 2015, Mehta et al., 2021).

Additionally, lead exposure triggered leukocytosis (WBC: 16.83 ± 0.35 ×10³/µL) and thrombocytosis (PLT: 615.33 ± 237.79 ×10³/µL), both significantly elevated compared to the control group (WBC: 4.50 ± 0.30 ×10³/µL; PLT: 184.33 ± 10.69 ×10³/µL). These hematological alterations are indicative of systemic inflammation and compensatory erythropoiesis, which are commonly observed in lead toxicity (Dongre et al., 2011). Treatment with *A. paniculata* effectively normalized these parameters, with Group V showing a reduction in WBC (8.33 ± 0.87 ×10³/µL) and PLT (396.67 ± 178.63 ×10³/µL). These findings suggest that APexerts anti-inflammatory effects, possibly through its ability to downregulate pro-inflammatory cytokines and inhibit platelet aggregation (Owoade et al., 2021).

Lead is known to induce oxidative stress, leading to lipid peroxidation of erythrocyte membranes, increased fragility, and hemolysis (Wani et al., 2015). The antioxidant-rich composition of *A. paniculata* may counteract these effects by scavenging reactive oxygen species (ROS) and promoting erythrocyte membrane stability (Rammohan et al., 2008). Furthermore, APmay facilitate lead excretion through renal pathways, as evidenced by the reduced blood lead levels in Group V. However, the transient increase in BLL observed in Groups III and IV may suggest initial redistribution of lead from tissues into circulation, a phenomenon frequently reported in detoxification therapies (Sanders et al., 2010).

Overall, these findings underscore AP’s dual role as a potent hematoprotective and detoxifying agent. By mitigating lead-induced oxidative damage, stabilizing erythropoiesis, and modulating inflammatory responses, AP demonstrates promising therapeutic potential in counteracting hematological toxicity associated with lead exposure.

4. Conclusion

This study demonstrates Andrographis paniculata’s efficacy in mitigating lead-induced hematological toxicity, particularly at 300 mg/kg. Its antioxidant, anti-inflammatory, and detoxifying properties position it as a promising adjuvant therapy for lead poisoning. Future studies should explore longer treatment periods and molecular markers (e.g., ALAD activity, oxidative stress enzymes) to elucidate AP’s mechanisms.

Ethical approval:

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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