**EFFECT OF AQUEOUS EXTRACT OF *Vernonia amygdalina* ON RENAL FUNCTION IN WISTAR ALBINO RATS EXPOSED TO LEAD TOXICITY**

**ABSTRACT**

**Background:** Lead exerts toxicity through multiple mechanisms, notably via the generation of reactive oxygen species (ROS), which induce oxidative stress, damage cellular structures, and impair antioxidant defense systems. Phytochemical analyses have identified several bioactive compounds in *Vernonia amygdalina* that could counteract lead-induced nephrotoxicity.

**Aim:** This study aimed to investigate the potential protective effect of aqueous *Vernonia amygdalina* (Bitter leaf) extract (AVAE) on renal function in Wistar Albino rats exposed to lead toxicity.

**Methods:** The leaves of *V. amygdalina* (bitter leaf) from five stations were screened for phytochemicals and heavy metals (lead, mercury, arsenic and cadmium) using thin layer chromatography and GBC XplorAA atomic absorption spectrophotometer**.** A total ofThirty-five (35) wistar albino rats were divided into five (5) groups of seven rats per group. Group I (Negative control) received normal saline only for 28 days, Group II (Positive control) were exposed to 50mg/kg lead acetate for 2 weeks and received no treatment throughout the study. Group III and IV were exposed to 50mg/kg lead acetate for 2 weeks and treated with AVAE at 2000mg/kg and 400mg/kg body weight (high and low dose respectively) orally for 28 days. Group V were not exposed to lead but treated with AVAE at a high dose, orally for 28 days. The experimental design included biomarkers of renal injury (Kidney Injury Molecule-1, Cystatin C), oxidative stress (DNA damage via 8-OHdG), and traditional renal function tests (urea and creatinine). Phytochemical analysis revealed the presence of bioactive compounds, including flavonoids, alkaloids, and saponins, while heavy metal analysis quantified contaminants in some of the plant samples. Acute toxicity studies confirmed the safety of AVAE up to 2000 mg/kg body weight.

**Results:**

The results from the study showed that Significant differences were observed between the groups (p < 0.05). Group II (positive control) showed the highest KIM-1, Cystatin C and DNA damage levels (indicative of severe kidney injury), while Groups III and IV (treated with high and low dose AVAE) showed significant reductions in KIM-1, Cystatin C and DNA damage levels with Group III (high dose AVAE) showing better response compared to Group II (Positive control), Group I and V which showed normal levels for all biomarkers. Elevated urea and creatinine in Group II indicated kidney dysfunction. AVAE treatment (Groups III and IV) reduced urea and creatinine levels, with high-dose treatment (Group III) providing the most significant improvement.

**Conclusion:**

Results demonstrated that AVAE significantly reduced oxidative stress, improved renal biomarkers, and mitigated kidney damage in lead-exposed rats, particularly at the high dose. These findings support the potential therapeutic role of *Vernonia amygdalina* in managing lead-induced nephrotoxicity. Future studies could explore its mechanisms of action and applicability in clinical settings to address heavy metal toxicity.

**Keywords**: Heavy metal poisoning, redox homeostasis, *Vernonia amygdalina,* bioactive compounds

**1. INTRODUCTION**

Heavy metal poisoning results from the accumulation of toxic metals in soft tissues, leading to diverse physiological and biochemical impairments. While trace amounts of metals such as zinc, copper, and manganese are essential for human health, their excessive accumulation as well as exposure to non-essential metals like lead, mercury, cadmium, and arsenic can result in systemic toxicity. Environmental contamination via industrial emissions, polluted water and air, contaminated foodstuffs, and lead-based paints has increased the risk of chronic heavy metal exposure [1]. Their bioaccumulation may lead to diverse toxic effects affecting different body tissues and organ systems. The toxicity of heavy metals depends on the properties of the given metal, dose, route, duration of exposure (acute or chronic), and extent of bioaccumulation. The detrimental impacts of heavy metals on human health are largely linked to their capacity to interfere with antioxidant defense mechanisms, primarily through their interaction with intracellular glutathione (GSH) or sulfhydryl groups (R-SH) of antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR), and other enzyme systems (Balali-Mood et al., 2021; Jomova et al., 2025).

Among these metals, lead (Pb) is particularly concerning due to its environmental persistence and high human toxicity. Though naturally occurring, anthropogenic activities such as mining, smelting, battery manufacturing, and pigment production have significantly elevated its levels in the environment [2],[3]. Pb is one of the major environmental and nondegradable pollutants, and overexposure to it results in several physiological and biochemical changes associated with liver and renal dysfunction. Pb exposure causes renal toxicity and hepatotoxicity through its ability to induce oxidative stress, resulting in an imbalance between the production of reactive oxygen species (ROS) and the potency of antioxidant defense enzymes. Pb exposure promotes ROS production that attacks biologically essential molecules, such as membrane lipids, proteins, DNA, and enzymes, and results in renal and hepatic dysfunction (Abdelrazek et al., 2022). Exposure to lead primarily occurs through ingestion or inhalation of contaminated food, water, dust, and tobacco products. In recognition of its public health burden, lead has been listed among the top 10 chemicals of major public health concern by the World Health Organization [4] and ranked second in toxicity on the ATSDR Substance Priority List [5].

Lead exerts toxicity through multiple mechanisms, notably via the generation of reactive oxygen species (ROS), which induce oxidative stress, damage cellular structures, and impair antioxidant defense systems [6],[7]. Although not a classical Fenton metal, lead can still contribute to ROS formation and interfere with redox homeostasis by depleting glutathione and inhibiting key antioxidant enzymes. Other toxicological mechanisms include binding to sulfhydryl groups on proteins, disrupting calcium and zinc-dependent processes, altering gene expression, and impairing DNA repair [8],[9].

Once absorbed, lead binds to erythrocyte proteins and is distributed to various tissues, including the brain, liver, kidneys, and bones. In the bloodstream, it has a biological half-life of around 30 days but can remain stored in bones for decades, potentially being remobilized during physiological states like pregnancy, menopause, or prolonged immobilization [10]. The kidneys play a crucial role in lead excretion through glomerular filtration and tubular secretion, though some lead is reabsorbed. Renal clearance rates vary depending on kidney function, typically ranging from 1 to 3 mL/min [11].

Given these mechanisms, the kidney emerges as a primary target organ in lead toxicity. Consequently, agents that mitigate renal oxidative damage are of significant therapeutic interest. One such promising agent is Vernonia amygdalina, commonly known as bitter leaf, a widely consumed medicinal plant in sub-Saharan Africa. A perennial shrub of the Asteraceae family, V. amygdalina is characterized by its intensely bitter taste, attributable to phytochemicals such as sesquiterpene lactones, flavonoids, alkaloids, and glycosides [12]. Traditionally used to treat gastrointestinal disorders, malaria, fever, and diabetes, bitter leaf is gaining scientific validation for its pharmacological properties, including antioxidant, anti-inflammatory, and nephroprotective effects [13].

Phytochemical analyses have identified several bioactive compounds in V. amygdalina that could counteract lead-induced nephrotoxicity. These include luteolin, apigenin, and various polyphenols capable of scavenging ROS, enhancing antioxidant enzyme activity, and protecting renal tissue integrity [14]. Given these properties, V. amygdalina presents a viable candidate for mitigating oxidative stress and tissue damage induced by heavy metal exposure.

This study therefore, aims to investigate the protective effect of Vernonia amygdalina extract against lead-induced renal toxicity, contributing to the growing body of evidence supporting its use in environmental toxicology and phytomedicine.

**2. MATERIALS AND METHODS**

**2.1 Collection and Identification of *V. Amygdalina* leaves**

Fresh leaves of *Vernonia amygdalina* were harvested from four (4) different stations (Eagle Island, Choba, Agip-Mgbuoshimini and Diobu) in Port Harcourt Metropolis, Port Harcourt, Nigeria. Identification and authentication of the leaves were conducted at the Herbarium, Department of Plant Science and Biotechnology, Rivers State University Nkpolu/Oroworokwo, Port Harcourt, with the Accession number RSU PbH0128.

**2.2 Preparation of Aqueous Extracts**

The leaves were washed with deionized water, sliced, and air dried using a fume cupboard for three weeks. They were macerated into fine powder to a constant weight of 317.26g. The resulting powder was soaked in 1000 mls of deionized water for 72 hours (3 days) with constant agitation. The solution was filtered with Whatman No.1 filter paper and the filtrates were concentrated by evaporation at 50°C in a water bath with further dryness in a Gallenkamp oven at 80°C until a constant weight was obtained. The yield of the crude aqueous extract obtained weighed 92.53g and was transferred into sterile sample bottle and refrigerated at 4oC before use for the study.

**2.3 Phytochemical and Heavy Metals Screening**

The leaves of *Vernonia amygdalina* (bitter leaf) from five (5) stations were screened for phytochemicals and heavy metals (lead, mercury, arsenic and cadmium) using thin layer chromatography and GBC XplorAA atomic absorption spectrophotometer instrument (made in Australia) as stated in the Operational Manual (GBC 2016) respectively.

**2.4 Laboratory Animals**

Fifty-six (56) albino rats were purchased from the Animal Breeding Unit, Department of Animal and Environmental Biology, Rivers State University, Port Harcourt. The animals were kept in cages to acclimatize for two weeks in conditions of ambient temperature 26-28°C, adequate ventilation, feeding with standard growers mash and clean water *ad libitum* throughout the study.

**2.5 LD­50 Determination for Lead Acetate**

Acute toxicity study (LD­­50) for lead acetate was determined by a method described by Lorke’s [15]. Nine (9) animals were divided into three groups of three animals each. Each group of animals were administered different doses (25mg/kg, 50mg/kg and 75mg/kg) of lead acetate intraperitoneally for two (2) weeks. The animals were placed under observation for two (2) weeks to monitor their behavior as well as mortality. Mortalities were observed in the group administered with 75mg/kg. Hence, the second phase did not proceed. The others were anaesthetized with chloroform and sacrificed. Whole blood was collected by cardiac puncture and centrifuged after clotting at 5000 rpm for five (5) minutes to obtain serum. Serum electrolyte, urea and creatinine concentrations were analyzed using MELA – 1A Electrolyte Analyzer, with serial no. 202026008 Labomiz Scientific Limited United States, Diacetylmonoxime method (DAM) and Jaffe’s method, respectively. The selected dose for the study was taken as 50mg/kg because there was no mortality recorded. This is to achieve nephrotoxicity and also avoid mortalities before the end of the study.

**2.6 LD50 Determination for *Vernonia amygdalina* (Bitter leaf) (Acute Toxicity)**

Acute oral toxicity of *Vernonia Amygdalina* was carried out using wistar albino rats by a method described by Lorke’s [15]. Nine (9) animals were divided into three groups of three (3) animals each. Each group of animals were administered different doses (200mg/kg, 400mg/kg and 600mg/kg) of aqueous extract of *V. amygdalina* orally for two (2) weeks. The animals were placed under observation for two (2) weeks to monitor their behavior as well as mortality [16]. No mortality was observed and the second phase proceeded, which was distributed into three groups of one (1) animal each. The animals were administered higher doses (1000mg/kg, 1500mg/kg and 2000mg/kg) of the aqueous extract and then observed for another two (2) weeks for behavior as well as mortality [16]. No mortality was also observed at the end of the second phase. Hence, doses of 2000mg/kg body weight and 400mg/kg body weight were chosen as high and low dose, respectively for the study.

**2.7 Sample Collection and Analysis**

At the end of the experiment, the animals were anaesthetized with chloroform and sacrificed. Whole blood was collected by cardiac puncture and centrifuged after clotting at 5000 rpm for five (5) minutes to obtain serum. The samples were assayed for Cystatic C (Cys-C), Kidney injury molecule-1 (Kim-1), DNA damage (8 Hydroxydeoxyguanosine (8 OHdG)), urea and creatinine using Sandwich ELISA technique, Diacetylmonoxime method (DAM) and Jaffe’s method respectively. The reagents were purchased through BRIDGE BID TECHNOLOGY with Production name (Rat Cystatin C ELISA Kit), Document number (ELAB-EK-WO12HR2L5439), Lot No. (WO12HR2L5439), Production name (Rat Kidney Injury Molecule 1), Document number (ELAB‑EK‑WO110TPJ4031), Lot No. (WO110TPJ4031) and Production name (8-Hydroxydeoxyguanosine ELISA Kit) Document number (ELAB-EK-FU00FD8Z2877), Lot No. (FU00FD8Z2877) respectively.

**2.8 Statistical Analysis**

Statistical analysis of the results was performed using Graphpad\_Prism (version 9.0.2.263) employing one-way analysis of variance (ANOVA)followed by Post Hoc test (Turkey, HSD) of multiple comparison for significance. Significance was set at (P < 0.05). Results are presented as Mean ± standard deviation.

**3. RESULTS**

**Table 1: Quantitative Analysis of Phytochemical Components in Aqueous Extract of *Vernonia amydalina* (Bitter leaf)**

|  |  |
| --- | --- |
| Components | *Vernonia Amygdalina* (mg/ml) |
| 1 Saponins | **5.97,5.97,5.97** |
| 2 Flavonoid | **4.89,4.89,4.89** |
| 3 Tanins | **7.62,7,62,7.62** |
| 4 Alkaloids | **8.26,8.26,8.26** |
| 5 Steroids | **0.15,0.15,0.15** |
| 6 Anthraquinones | **0.14,0.14,0.14** |
| 7 Phenol | **0.78,0.78,0.78** |
| 8 Glycoside | **0.41,0.41,0.41** |

*The result shows that AVAE contains bioactive compounds with potential medicinal properties, especially those relevant to antioxidant, anti-inflammatory, and nephroprotective effects such as alkaloids, saponins, tannins, flavonoids and phenols with alkaloids having the highest concentration.*

**Table.2 Heavy Metal Analysis of *Vernonia amygdalina* leaves Extract**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Samples | Lead (Pb)  (mg/kg) | Mercury (Hg)  (mg/kg) | Arsenic (As)  (mg/kg) | Cadmium (Cd)  (mg/kg) |
| Eagle Island | 17.4 | 0.03 | 9.00 | 6.5 |
| Choba | 11.1 | <0.01 | 3.2 | 1.12 |
| Agip (Mgbuoshimini) | 15.3 | 0.87 | 7.5 | 4.7 |
| Diobu | 13.4 | 0.06 | 5.8 | 2.8 |

*The results displayed in table 2 presents the concentrations of lead (Pb), Mercury (Hg), Arsenic (As) and Cadmium (Cd) in the leaf extracts from four different locations (Eagle Island, Choba, Agip [Mgbuoshimini] and Diobu). Although there are measurable levels of these metals, the values for Choba falls within acceptable limits for medicinal use, which was used in the course of this study.*

**Table 3: Mean ± SD levels of Kim-1, Cystatin C and DNA Damage for all Experimental Groups**

|  |  |  |  |
| --- | --- | --- | --- |
| **Groups/Parameters** | **Kim-1**  **(0 – 500pg/ml)** | **Cys C**  **(0 – 20ng/ml)** | **DNA Damage**  **(0 – 00ng/ml)** |
| **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| Group 1 (NC) (N – 7) | 2.93 ± 2.930 | 0.92 ± 0.074 | 1.94 ± 0.105 |
| Group 2 (PC-(Lead induced)  (N – 7) | 699.90 ± 0.400**1** | 40.06 ± 0.111 | 199.90 ± 0.416 |
| Group 3(V.A 2000mg) (N – 7)  Lead induced | 21.12 ± 0.257 | 1.94 ± 0.245 | 11.18 ± 0.257 |
| Group 4 (V.A, 400mg) (N – 7)  Lead induced | 50.11 ± 0.154 | 5.07 ± 0.159 | 29.95 ± 0.315 |
| Group 5 (No lead induced.  High dose treatment of V.A only) (N – 7) | 3.035 ± 0.122 | 0.93 ± 0.115 | 1.92 ± 0.152 |
| P –value | <0.0001 | <0.0001 | <0.0001 |
| F-value | 11633829 | 86775 | 679128 |
| **Remark** | S | S | S |

*The result shows the Mean ± SD levels of Kidney Injury Molecule-1 (Kim-1), Cystatin C (Cys C) and DNA Damage. The result shows that lead‑exposed rats without treatment (Group II) had dramatically elevated kidney injury markers Kim‑1 (~700 pg/mL), Cystatin C (~40 ng/mL), and DNA damage (~200 ng/mL) compared to the controls (all biomarkers ≈1–3), while AVAE treatment dose‑dependently lowered these markers, with the high dose (Group III) showing the greatest reduction toward normal levels.*

***Key:*** *NC - Negative Control, PC - Positive Control,**V.A - Vernonia amygdalina, S - Significant*

**Table 4: Mean ± SD levels of Urea and Creatinine for all Experimental Groups**

|  |  |  |
| --- | --- | --- |
| **Groups/Parameters** | **Urea**  **(0.6 – 1.4 mmol/L)** | **Creatinine**  **(18 – 62umol/L)** |
| **Mean ± SD** | **Mean ± SD** |
| Group 1 (NC) (N – 7) | 0.63 ± 0.018 | 18.09 ± 0.083 |
| Group 2 (PC-(Lead induced) (N – 7) | 1.61 ± 0.014 | 70.12 ± 0.139 |
| Group 3 (V.A 2000mg) (N – 7)  Lead induced | 0.82 ± 0.016 | 26.04 ± 0.133 |
| Group 4 (V.A, 400mg) (N – 7)  Lead induced | 1.00 ± 0.063 | 30.05 ± 0.239 |
| Group 5 (No lead induced.  High dose treatment of V.A only)  (N – 7) | 0.62 ± 0.033 | 18.17 ± 0.154 |
| P –value | <0.0001 | <0.0001 |
| F-value | 959.6 | 130630 |
| Remark | S | S |

***The Mean ± SD levels of urea and creatinine for all experimental group shows that untreated lead exposure (Group II) caused a significant rise in traditional renal function tests urea (~1.61 mmol/L) and creatinine (~70 µmol/L) compared to the controls (urea ~0.63 and creatinine ~18), and AVAE treatment reduced both parameters, with the 2000 mg/kg dose (Group III) achieving values closest to control***

***Key:*** *NC - Negative Control, PC - Positive Control, V.A - Vernonia amygdalina, S - Significant*

**Table.5: Tukey's Multiple Comparisons Test Results for Kidney Injury Molecule-1 for all Experimental Groups**

|  |  |  |
| --- | --- | --- |
| **Tukey's Multiple Comparisons Test** | **p-value** | **Remark** |
| Group 1 KIM-1 vs. Group 2 KIM-1 | <0.0001 | S |
| Group 1 KIM-1 vs. Group 3 KIM-1 | <0.0001 | S |
| Group 1 KIM-1 vs. Group 4 KIM-1 | <0.0001 | S |
| Group 1 KIM-1 vs. Group 5 KIM-1 | 0.9360 | NS |
| Group 2 KIM-1 vs. Group 3 KIM-1 | <0.0001 | S |
| Group 2 KIM-1 vs. Group 4 KIM-1 | <0.0001 | S |
| Group 2 KIM-1 vs. Group 5 KIM-1 | <0.0001 | S |
| Group 3 KIM-1 vs. Group 4 KIM-1 | <0.0001 | S |
| Group 3 KIM-1 vs. Group 5 KIM-1 | <0.0001 | S |
| Group 4 KIM-1 vs. Group 5 KIM-1 | <0.0001 | S |

*The result shows via Tukey’s post‑hoc test that all pairwise comparisons of Kim‑1 between groups were statistically significant (p < 0.0001) except between the negative control and the AVAE‑only group (p = 0.9360), confirming that AVAE alone does not alter Kim‑1 but that it significantly mitigates the lead‑induced rise in Kim‑1.*

***Key:*** *S - Significant, NS - Not Significant*

**Table.6: Tukey's Multiple Comparisons Test Results for Cystatin C for all Experimental Groups**

|  |  |  |
| --- | --- | --- |
| **Tukey's Multiple Comparisons Test** | **Adjusted P Value** | **Remark** |
| Group 1 Cys C vs. Group 2 Cys C | **<0.0001** | S |
| Group 1 Cys C vs. Group 3 Cys C | **<0.0001** | S |
| Group 1 Cys C vs. Group 4 Cys C | **<0.0001** | S |
| Group 1 Cys C vs. Group 5 Cys C | **>0.9999** | NS |
| Group 2 Cys C vs. Group 3 Cys C | **<0.0001** | S |
| Group 2 Cys C vs. Group 4 Cys C | **<0.0001** | S |
| Group 2 Cys C vs. Group 5 Cys C | **<0.0001** | S |
| Group 3 Cys C vs. Group 4 Cys C | **<0.0001** | S |
| Group 3 Cys C vs. Group 5 Cys C | **<0.0001** | **S** |
| Group 4 Cys C vs. Group 5 Cys C | **<0.0001** | **S** |

*The result shows that Cystatin C levels followed the same pattern: every comparison involving the lead‑exposed group versus any other was significant (p < 0.0001), while the negative control versus the AVAE‑only group was not (p > 0.9999), demonstrating specific protection by AVAE against lead‑induced Cystatin C elevation*.

***Key:*** *S - Significant, NS - Not Significant*

**Table 7: Tukey's Multiple Comparisons Test Results for DNA Damage for all Experimental Groups**

|  |  |  |
| --- | --- | --- |
| **Tukey's Multiple Comparisons Test** | **Adjusted P Value** | **Remark** |
| Group 1 DNA D vs. Group 2 DNA D | <0.0001 | S |
| Group 1 DNA D vs. Group 3 DNA D | <0.0001 | S |
| Group 1 DNA D vs. Group 4 DNA D | <0.0001 | S |
| Group 1 DNA D vs. Group 5 DNA D | >0.9999 | NS |
| Group 2 DNA D vs. Group 3 DNA D | <0.0001 | S |
| Group 2 DNA D vs. Group 4 DNA D | <0.0001 | S |
| Group 2 DNA D vs. Group 5 DNA D | <0.0001 | S |
| Group 3 DNA D vs. Group 4 DNA D | <0.0001 | S |
| Group 3 DNA D vs. Group 5 DNA D | <0.0001 | S |
| Group 4 DNA D vs. Group 5 DNA D | <0.0001 | S |

*The result shows that DNA damage (8‑OHdG) comparisons mirrored those of Kim‑1 and Cystatin C: all lead‑involving contrasts were significant (p < 0.0001), whereas the negative control versus AVAE‑only was not, indicating AVAE prevents lead‑induced oxidative DNA damage.*

**Key:** *S - Significant, NS - Not Significant*

**Table 8: Tukey's Multiple Comparisons Test Results for Urea for all Experimental Groups**

|  |  |  |
| --- | --- | --- |
| **Tukey's Multiple Comparisons Test** | **Adjusted P Value** | **Remark** |
| Group 1 Urea vs. Group 2 Urea | <0.0001 | S |
| Group 1 Urea vs. Group 3 Urea | <0.0001 | S |
| Group 1 Urea vs. Group 4 Urea | <0.0001 | S |
| Group 1 Urea vs. Group 5 Urea | 0.9853 | NS |
| Group 2 Urea vs. Group 3 Urea | <0.0001 | S |
| Group 2 Urea vs. Group 4 Urea | <0.0001 | S |
| Group 2 Urea vs. Group 5 Urea | <0.0001 | S |
| Group 3 Urea vs. Group 4 Urea | <0.0001 | S |
| Group 3 Urea vs. Group 5 Urea | <0.0001 | S |
| Group 4 Urea vs. Group 5 Urea | <0.0001 | S |

*The result shows that urea comparisons also demonstrated significant differences whenever lead exposure was involved (p < 0.0001) but no difference between negative control and AVAE‑only, confirming AVAE’s specificity in normalizing urea levels after lead toxicity.*

**Key:** *S - Significant, NS - Not Significant*

**Table 9: Tukey's Multiple Comparisons Test Results for Creatinine for all Experimental Groups**

|  |  |  |
| --- | --- | --- |
| **Tukey's Multiple Comparisons Test** | **Adjusted P Value** | **Remark** |
| Group 1 Cr vs. Group 2 Cr | <0.0001 | S |
| Group 1 Cr vs. Group 3 Cr | <0.0001 | S |
| Group 1 Cr vs. Group 4 Cr | <0.0001 | S |
| Group 1 Cr vs. Group 5 Cr | 0.8745 | NS |
| Group 2 Cr vs. Group 3 Cr | <0.0001 | S |
| Group 2 Cr vs. Group 4 Cr | <0.0001 | S |
| Group 2 Cr vs. Group 5 Cr | <0.0001 | S |
| Group 3 Cr vs. Group 4 Cr | <0.0001 | S |
| Group 3 Cr vs. Group 5 Cr | <0.0001 | S |
| Group 4 Cr vs. Group 5 Cr | <0.0001 | S |

*The result shows the same for creatinine: significant differences for all lead‑exposed versus other groups (p < 0.0001), with no change between negative control and AVAE‑only, reinforcing AVAE’s effectiveness in preserving normal renal function under lead challenge.*

***Key:*** *S - Significant, NS - Not Significant*

**4. DISCUSSION**

Lead toxicity presents a significant environmental and health hazard due to its wide-ranging biological effects, including renal dysfunction, oxidative stress, and alterations in enzymatic activities within both human and animal organisms. This study investigates the effects of aqueous *Vernonia amygdalina* extract on the renal function of nephrotoxic wistar albino rats induced with lead acetate. The findings from this study underscore the significant potential of *Vernonia amygdalina* (bitter leaf) as a therapeutic agent against lead-induced nephrotoxicity.

The phytochemical analysis (Table 1) revealed that *V. amygdalina* is rich in alkaloids, flavonoids, tannins, saponins, and other bioactive compounds. These compounds are known for their antioxidant, anti-inflammatory, and nephroprotective properties, consistent with the studies of [13]. Interestingly, the heavy metal analysis (Table 2) indicated that while *V. amygdalina* possesses some measurable levels of heavy metals, some of which were within acceptable thresholds for medicinal use. This suggests that proper preparation minimizes potential toxicity risks, aligning with regulatory guidelines for medicinal plants [17].

In this study, lead exposure in rats (Group II) produced a marked increase in renal injury biomarkers Kidney Injury Molecule-1 (Kim-1), Cystatin C, and oxidative DNA damage marker 8-OHdG as well as elevated serum urea and creatinine, confirming the development of lead-induced nephrotoxicity (Tables 3 and 4).

The therapeutic intervention with aqueous *Vernonia amygdalina* extract (AVAE) significantly reversed these alterations, especially at the high dose (2000 mg/kg), indicating dose-dependent nephron-protection. Kim-1 and Cystatin C are early, sensitive biomarkers of renal tubular injury and dysfunction. The significantly lower levels of these biomarkers in AVAE-treated groups (III and IV), compared to the lead-only group (II), point to a protective or reparative effect of the extract on kidney epithelial cells (Table 3), consistent with earlier reports on the nephron-protective effects of polyphenolic-rich plant extracts [13],[14]. Tukey's post-hoc analysis further confirmed these findings, showing significant group differences in Kim-1 and Cystatin C levels (Tables 5 and.6).

Furthermore, the normalization of 8-OHdG, a widely recognized marker of oxidative DNA damage, suggests that AVAE may attenuate reactive oxygen species (ROS) mediated injury, likely due to its rich phytochemical profile including flavonoids, tannins, alkaloids, and phenolic compounds (Table 1). These compounds are known to exert antioxidant effects by scavenging free radicals, chelating metal ions, and enhancing the activity of endogenous antioxidant enzymes [8],[12]. The statistically significant reductions in DNA damage observed in AVAE-treated animals, particularly in the high-dose group (Group III), were validated by the multiple comparison test results (Table 7).

The reduction in traditional renal function markers (urea and creatinine) further reinforces the extract’s renal protective potential. These improvements reflect not only biochemical recovery but also functional restoration of glomerular filtration, which is often compromised in lead toxicity due to vascular and cellular injury in nephrons (Table 4). Post-hoc results support these observations by revealing significant differences among groups (Tables 8 and 9).

Interestingly, the AVAE only group (Group V), which was not exposed to lead, exhibited biomarker levels indistinguishable from the negative control (Group I). This finding confirms the extract’s biocompatibility and safety, corroborated by acute toxicity tests showing no lethality or adverse effects even at the highest tested dose (2000 mg/kg). Thus, AVAE appears not to pose inherent nephrotoxic risks at therapeutic doses, highlighting its suitability for use in chronic treatment models or preventive medicine. These results suggest that *Vernonia amygdalina* may exert its protective effect through multiple pathways, antioxidant defense, anti-inflammatory activity and potential chelation of lead ions. These findings align with previous pharmacological studies indicating the efficacy of *V. amygdalina* in ameliorating chemically induced organ damage [13].

**CONCLUSION**

This study demonstrates that *Vernonia amygdalina* possesses significant protective effects against lead-induced nephrotoxicity in wistar albino rats. The extract effectively reduced renal dysfunction, oxidative stress and inflammation, with higher doses showing greater efficacy. These findings support the ethnomedicinal use of *Vernonia amygdalina* for managing heavy metal (lead) toxicity and provide a foundation for its development into a therapeutic agent.

**Ethical Approval**

Animal Ethic committee approval has been collected and preserved by the author(s)

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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