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Chelation Potential of Aqueous Leaf Extracts of *Vernonia amygdalina* and *Phyllanthus amarus* on Kidney Functions in Lead-intoxicated Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the study was to investigate the chelation potential of aqueous leaf extracts of *Vernonia amygdalina* and *Phyllanthus amarus* in albino wistar rats intoxicated with lead using kidney function markers as indicators, and compare results to an EDTA chelation therapy. Both plants were assessed for phytochemicals. Forty-two male albino wistar rats with mean weight of 75 g were divided into seven groups of six animals each: normal NC (normal saline) and intoxicated control IC (1000 ppm lead acetate in water) for three weeks; Drug treated control DTC (75 mg/kg Na_2EDTA co-administered with 5 mg/kg Calcium); *V. amygdalina*-treated IT1 (100 mg/kg) and IT2 (200 mg/kg); *P. amarus*-treated IT3 (100 mg/kg) and IT4 (200 mg/kg) for eight consecutive days after three weeks of exposure. Biomarkers analyzed include serum concentrations of urea, creatinine, and electrolytes. Histological assessments on kidney tissues were performed. A significantly elevated *($p < 0.05$)* trend was detected in serum urea (17.93±2.99 mmol/L) and creatinine

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(4.28±1.38 mg/dL) levels of the intoxicated control IC. The DTC (10.16±1.46 mmol/L), 100 mg/kg *VA*(12.69±1.59 mmol/L), 200 mg/kg *VA*(10.48±1.12 mmol/L), 100 mg/kg *PA*(9.96±1.83 mmol/L) and 200 mg/kg *PA* (9.12±1.19 mmol/L) treatment groups showed significant *($p < 0.05$)* decrease in serum urea concentrations close to NC (10.55±1.20 mmol/L). Similar significant reversal *($p < 0.05$)* of the elevated serum creatinine levels were observed in DTC (2.60±1.08 mg/dL), 100 mg/kg *VA*(2.22±0.43 mg/dL), 200 mg/kg *VA*(2.07±0.416 mg/dL), 100 mg/kg *PA*(1.99±0.27 mg/dL) and 200mg/kg *PA* (2.04±0.28 mg/dL). The intoxicated control IC exhibited remarkable decline ($p < 0.05$) in serum sodium (69.67±8.59 mmol/L) and potassium (4.93±0.71 mmol/L) concentrations. These were significantly elevated ($p < 0.05$) in all treatment groups. Histological examinations confirmed the amelioration of deranged tissues in the treatment groups. Aqueous leaf extracts of *V. amygdalina* and *P. amarus* can compete favorably with an EDTA chelation therapy, with regard to reversing impaired kidney functions as a result of lead intoxication.

Keywords: Lead; chelation; V. amygdalina; P. amarus; kidney.

1. INTRODUCTION

Lead has been associated with various forms of cancer, nephrotoxicity, central nervous system effects and cardiovascular diseases in human [1]. Excess lead is known to reduce the cognitive development and intellectual performance in children and to increase blood pressure and cardiovascular diseases incidence in adults [2]. Lead poisoning typically results from ingestion of food or water contaminated with lead, but may also occur after accidental ingestion of contaminated soil, dust, or lead-based paint [3]. A series of lead poisonings in Zamfara State, a poor state blessed with abundant mineral resources, Nigeria, led to the deaths of at least 163 people between March and June 2010 [4]. The lead poisoning remained the cause of high mortality of children which was initially attributed to malaria [4].

Many heavy metals, including lead are known to induce over-production of reactive oxygen species (ROS), which are the by-products of many degenerative reactions in many tissues, hence will affect the regular metabolism by damaging the cellular components [5]. In addition, ROS are highly reactive to membrane lipids, protein and DNA. They are believed to be the major contributing factors to stress injuries and to cause rapid cellular damage [6]. Oxidative stress has been reported as a major mechanism of lead induced toxicity. Under the influence of lead, onset of oxidative stress occurs on account of two pathways which operate simultaneously: first, the generation of ROS, like hydroperoxides, singlet oxygen and hydrogen peroxide; and second, the depletion of antioxidant reserves [7]. Lead exposure has also been shown to exhibit enhanced rate of liver lipid peroxidation and alter antioxidant defense systems in rats [8].

In most studies of chelating agents in treating cases of metal intoxication, focus has been primarily on the mobilization (mainly due to renal excretion) of toxic metal. Whereas the net ionic charge of the chelator defines its absorption, distribution and ability to reach the metal ion for binding; the net ionic charge of the complex decides its elimination from the specific site and excretion from the body [9]. Several chelating agents are available, having different affinities for different metals. Synthetic chelators such as EDTA, DMPS, DMSA, and other drugs are known to be toxic to the kidneys, in particular [10]. The chelating process also removes vital nutrients such as vitamins C and E, therefore these must be supplemented. Problems with chelation therapy have become a serious problem today for two main reasons: First, many people are already extremely deficient in many essential minerals such as zinc, chromium, selenium, manganese and others; also, removal of some essential minerals can further upset the body chemistry, often in ways that are difficult to repair [11,12].

Plants form the main ingredients of medicine in traditional system of healing and have been the source of inspiration for several major pharmaceutical drugs [13]. *Vernonia amygdalina,* commonly called “bitter leaf” or *onugbu (igbo),* grows in a range of ecological zones in Africa, produces large mass forage and is drought tolerant [14]. *V. amygdalina* has been discovered to produce a variety of phytochemicals and bitter sesquiterpene lactones which contribute to the diverse bioactivities of the plant [15,16] and provide anti-oxidant benefits [17]. Some peptides (edotides) isolated from aqueous *V. amygdalina* is currently being considered as alternative to tamoxiten, having shown to be potent inhibitor of nitrogen activated protein kinases (NAPK) which

are crucial for breast tumour growth [18]. Ethanolic leaf extract of *V. amygdalina* has been found to induce apoptosis in mylegenous K562 leukaemic cell line [19].

Phyllanthus amarus, an ayurvedic remedy, commonly called 'stone-breaker' or *ite kwo nwa (igbo)* has been valued in many countries for its medicinal properties and curative potentials for a variety of ailments of the stomach, genitourinary system, liver, kidney and spleen [20]. According to Odetola and Akojenu [21], aqueous leaf extract of *P. amarus* demonstrated anti-diarrhea and gastro-intestinal protective potential in mice. Several reports have demonstrated its hypoglycemic effect [22], chemoprotective effect [23], antiviral [24] and antinociceptive effect [25].

In the course of inappropriate administration in response to heavy-metal toxicity, chelation therapy brings risk of cancer, neurodevelopmental disorder from toxicity and death [26]. This study therefore sets out to determine the chelation prospects of *Vernonia amygdalina* and *Phyllanthus amarus* on kidney functions of albino wistar rats intoxicated with lead, comparing the outcomes with an EDTA chelation therapy.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Vernonia amygdalina* and *Phyllanthus amarus* were collected from gardens in Eziobodo, Imo State, Nigeria and identified at the Herbarium unit of the Department of Forestry and Wildlife, Federal University of Technology Owerri, FUTO, Nigeria.

2.2 Preparation of Aqueous Extracts

The plant samples were separately air-dried at room temperature for two weeks and subsequently ground into fine powder using a mechanical grinder (Henman, Japan). A resulting powder weight of 100 g each was soaked in 2000 ml of distilled water for 24 hours. They were both filtered (Whatman No.1 paper) and the filtrates concentrated by evaporation at 50°C in a water bath with further dryness in a Gallenkamp oven at 80°C until constant weights were obtained.

2.3 Phytochemical Screening

The crude aqueous extracts of *Vernonia amygdalina* leaves and whole plant of *Phyllanthus amarus* were subjected to qualitative chemical screening for identification of various classes of active chemical constituents. The phytochemical analysis was done according to standard methods [27].

2.4 Laboratory Animals

Forty-two, six week old male albino rats with mean weight of 74.5 g were purchased from the Animal Breeding Unit, FUTO. The animals were kept in cages to acclimatize for two weeks to conditions of ambient temperature 26-28°C, adequate ventilation, feeding with standard growers mash and clean water *ad libitum.*

2.5 Induction of Lead Intoxication

1000 ppm of lead acetate in distilled water was given orally as drinking water to the intoxicated groups continuously for three (3) weeks, as modified [28].

2.6 Experimental Design

The animals were divided into seven groups of six rats per group. Animals in all groups, except the normal control, were exposed to the lead-intoxicated drinking water for the period of intoxication and orally treated once daily for eight days as follows:

- Non-intoxicated control received normal saline
- Intoxicated control received no treatment but normal saline
- EDTA-Drug treated control received 75 mg/kg b.w Na_2EDTA + 5 mg/kg Calcium
- Intoxicated treated IT 1 received 100 mg/kg *V. amygdalina*
- Intoxicated treated IT 2 received 200 mg/kg *V. amygdalina*
- Intoxicated treated IT 3 received 100 mg/kg *P. amarus*
- Intoxicated treated IT 4 received 200 mg/kg *P. amarus*

2.7 EDTA Drug-treatment

75 mg/kg/b.w Disodium EDTA (BDH, UK) was dissolved, co-administered with 5 mg/kg/b.w Calcium (as Calcium lactate pentahydrate, Meyer Organics, PVT Ltd, India) in normal saline and given orally by gavage.

2.8 Collection and Analysis of Samples

At the end of the experimental period, the animals were anaesthetized with dichloromethane and sacrificed, with ethical consent from the Center of Biochemistry and Scientific research unit. Whole blood was collected by cardiac puncture and was centrifuged after clotting at 5000 rpm for ten (10) minutes to obtain serum. Urea and creatinine concentrations were analyzed using Diacetylmonoxime method [29] and the Jaffe's reaction method by Seaton and Ali [30] respectively. Electrolytes were estimated with an automated ion selective electrode analyzer (BAUR-biloyte Spin 5, PCE-Zion). All chemicals and reagents used were of standard analytical grade.

2.9 Statistical Analysis

Data was collected and analysed using Statistical Package for Social Science (SPSS) software. All data was expressed as Mean±standard error of the mean (SEM) and the $p<0.05$ was considered significant. Graph pad prism 5.0 was used for graphical interpretation.

2.10 Histological Assessment

Whole kidneys were excised, sliced and fixed in 10% buffered formaldehyde solution for use in the histological examinations.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The percentage yields of *Vernonia amygdalina* and *Phyllanthus amarus* were 18.60% and 10.33% respectively. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins and cyanogenic glycosides as shown in 'Table 1'.

3.2 Effects of Aqueous *Vernonia amygdalina* and *Phyllanthus amarus* on Serum Urea Concentrations of Lead-intoxicated Rats

The effect of graded doses of aqueous *V. amygdalina* and *P. amarus* on serum urea mmol/L concentration of lead-intoxicated rats is demonstrated in 'Fig. 1'. The drug-treated control DTC (10.16±1.46), IT1 [100 mg/kg *VA*] (12.69±1.59), IT2 [200 mg/kg *VA*] (10.48±1.12), IT3 [100 mg/kg *PA*] (9.96±1.83) and IT4 [200 mg/kg *PA*] (9.12±1.19) treatment groups showed significant ($p < 0.05$) decrease in serum urea concentrations from observed significantly elevated ($p < 0.05$) levels of the intoxicated control (17.93±2.99). The treatment groups showed no significant change to the normal control (10.55±1.20).

Table 1. Qualitative phytochemical analysis of the plant samples

Phytochemical	*V. amygdalina*	*P. amarus*
Saponins	+	+
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Cyanogenic glycosides	+	+

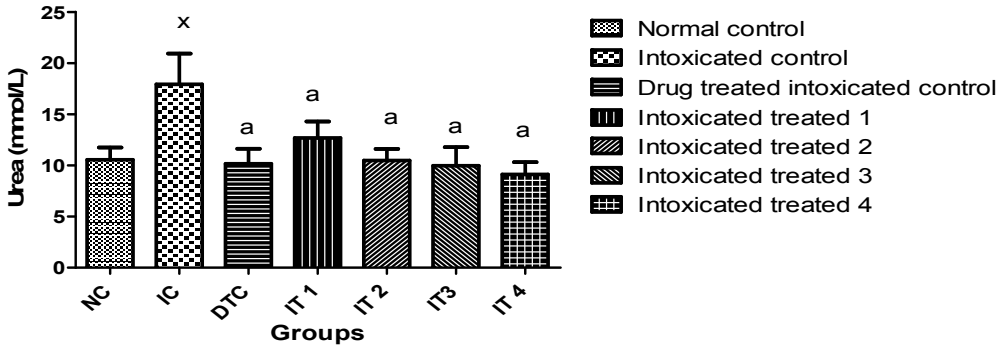
+ Indicates presence of compounds

3.3 Effects of Aqueous *Vernonia amygdalina* and *Phyllanthus amarus* on Serum Creatinine Concentrations of Lead-intoxicated Rats

'Fig. 2' illustrates the effects of graded doses of *V. amygdalina* and *P. amarus* on serum creatinine mg/dL concentration of lead-intoxicated rats. The DTC (2.60±1.08), IT1 [100 mg/kg *VA*] (2.22±0.43), IT2 [200 mg/kg *VA*] (2.07±0.416), IT3 [100 mg/kg *PA*] (1.99±0.27) and IT4 [200 mg/kg *PA*] (2.04±0.28) exhibited a significant reversal ($p < 0.05$) of the observed significant increase ($p < 0.05$) of serum creatinine levels in the intoxicated control IC (4.28±1.38). The treatment groups showed no significant variation to the normal control (1.97±0.17).

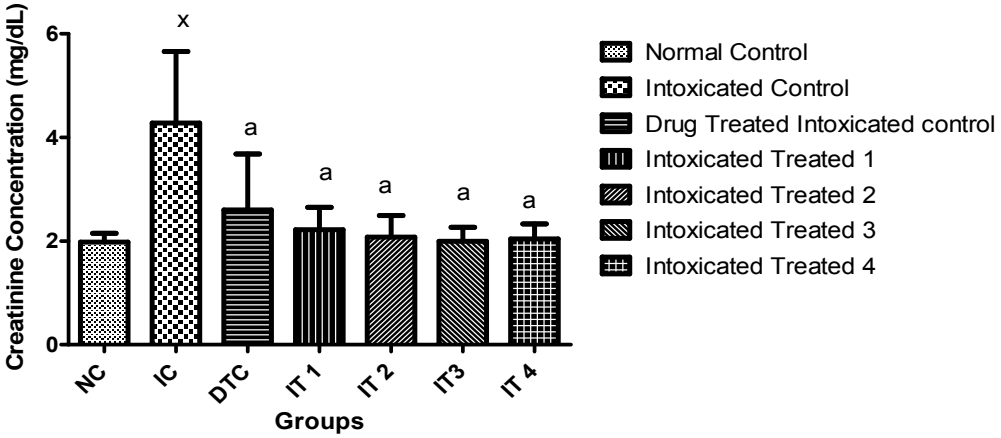
3.4 Effects of Aqueous *Vernonia amygdalina* and *Phyllanthus amarus* on Serum Sodium ion Concentration of Lead-intoxicated Rats

The effect of graded doses of *V. amygdalina* and *P. amarus* on serum sodium ion mmol/L concentration of lead-intoxicated rats is demonstrated in 'Fig. 3'. The intoxicated control (69.67±8.59) exhibited a significant decline ($p < 0.05$) in serum sodium concentrations compared to the normal control (125.81±3.31). Treatment groups DTC (134.0±1.50), IT1 [100 mg/kg *VA*] (128.30±2.16), IT2 [200 mg/kg *VA*] (126.0±4.60), IT3 [100 mg/kg *PA*] (131.20±1.83) and IT4 [200 mg/kg *PA*] (133.30±1.36) demonstrated significant increase ($p < 0.05$) in serum sodium ion levels and showed no significant change to the normal control.



Comparison of serum levels of different experimental groups.
Values are mean±SD, n = 6, P < 0.05.
x vs NC and a vs IC.

Fig. 1. Comparative effects of graded doses of aqueous *Vernonia amygdalina* and *Phyllanthus amarus,* and EDTA-drug control on serum urea concentration of lead-intoxicated rats

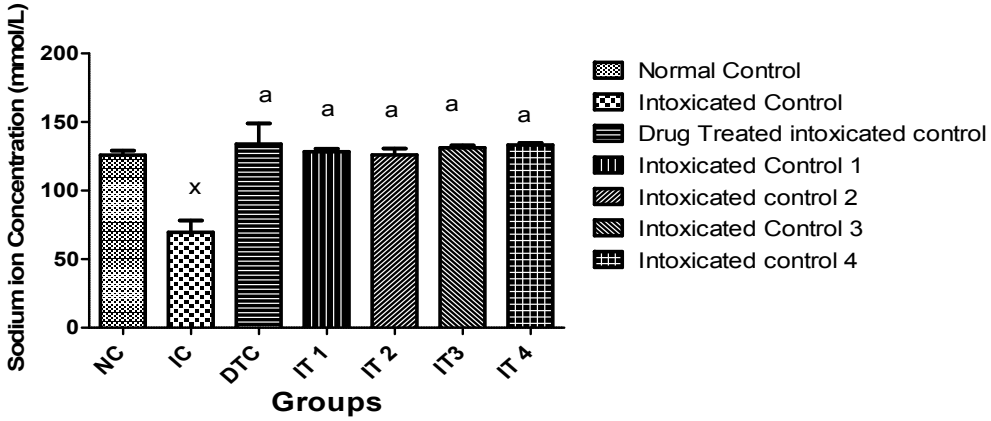


Comparison of serum creatinine levels of different experimental groups.
Values are mean±SD, n = 6, P < 0.05.
x vs NC and a vs IC

Fig. 2. Comparative effects of graded doses of aqueous *Vernonia amygdalina* and *Phyllanthus amarus,* and EDTA-drug control on serum creatinine concentration of lead-intoxicated rats

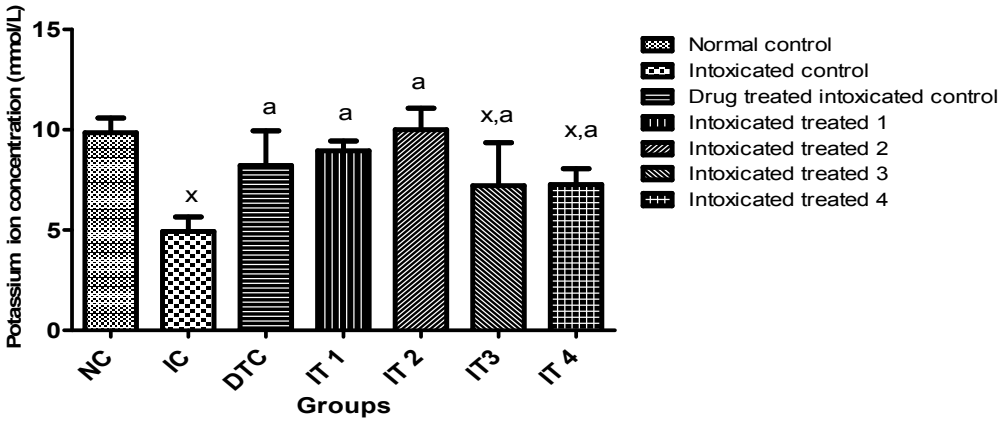
3.5 Effects of Aqueous *Vernonia amygdalina* and *Phyllanthus amarus* on Serum Potassium ion Concentration of lead-intoxicated Rats

'Fig. 4' shows the effects of graded doses of *V. amygdalina* and *P. amarus* on serum potassium ion mmol/L concentration of intoxicated rats. The intoxicated control IC (4.93±0.72) exhibited a significant decline in serum potassium levels compared to the normal control (9.85±0.74). The DTC (8.22±1.73), IT1 [100 mg/kg *VA*] (8.95±0.49), IT2 [200 mg/kg *VA*] (10.0±1.07), IT3 [100 mg/kg *PA*] (7.21±2.14) and IT4 [200 mg/kg *PA*] (7.27±0.80) exhibited significant increase *($p < 0.05$)* in serum potassium concentrations and showed no significant change to the normal control except groups IT3 and IT4 which revealed decrease from the normal control concentration.



Comparison of serum sodium ion levels of different experimental groups.
Values are mean±SD, n = 6, P < 0.005.
x vs NC and a vs IC.

Fig. 3. Comparative effects of graded doses of aqueous *Vernonia amygdalina* and *Phyllanthus amarus,* and EDTA-drug control on serum sodium ion concentration of lead-intoxicated rats



comparison of serum pottasium levels of different experimental groups. values are mean±SD, n=6, P < 0.05.
x vs NC and a vs IC

Fig. 4. Comparative effects of graded doses of aqueous *Vernonia amygdalina* and *Phyllanthus amarus,* and EDTA-drug control on serum potassium ion concentration of lead-intoxicated rats

3.6 Histological Assessment Results

In the intoxicated control, stromal proliferation with congestion, compressed tubules, shrunken glomeruli with some cystially dilated spaces containing eosinophilic materials were observed. However, few slightly enlarged tubules, slightly shrunken glomeruli and oedemation were observed in the DTC and groups treated with the aqueous plant extracts. These observations are shown in 'Fig. 5'.

4. DISCUSSION

Research evidence has shown that lead is a toxic agent with multiple target organs such as the hematopoietic system, immune system, kidneys and nervous system [31,28]. Administration of various antioxidants can prevent or subdue various toxic effects of lead and generation of oxidative stress [12]. Results of phytochemical analysis of both plants indicated the presence of alkaloids, flavonoids,

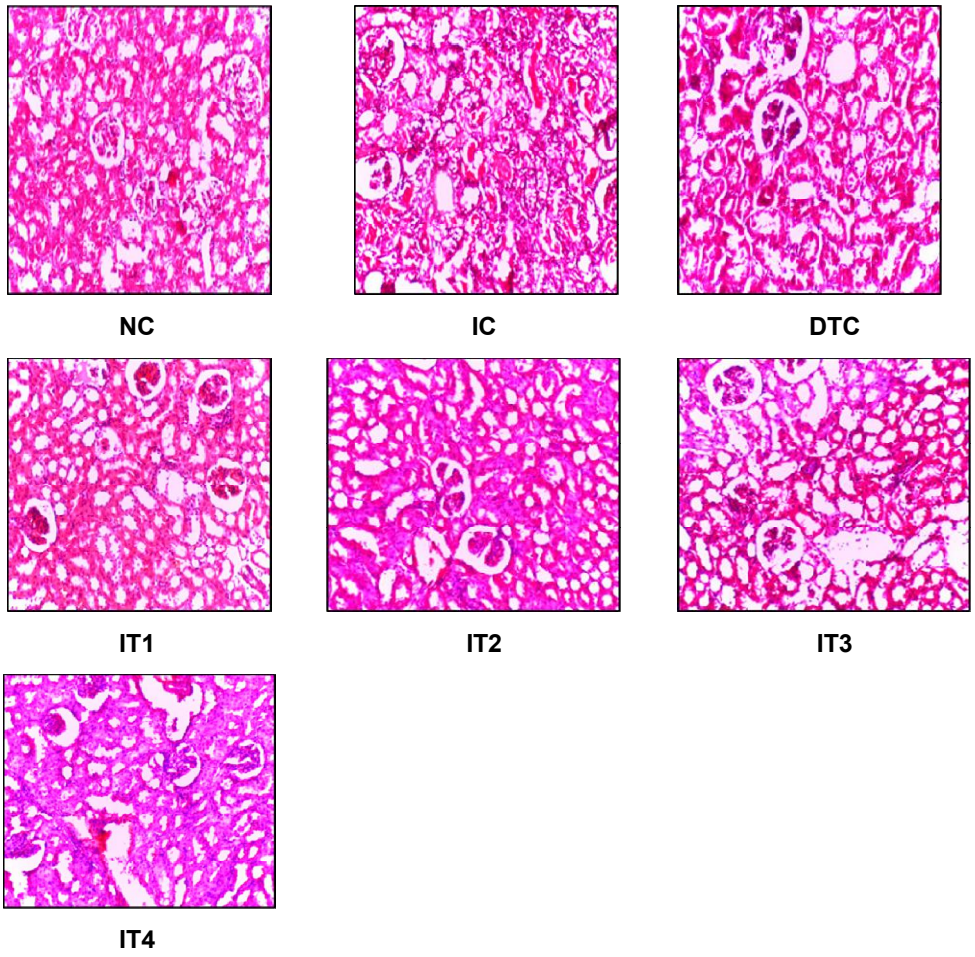


Fig. 5. Comparative kidney histopathological assessments of graded doses of aqueous *Vernonia amygdalina* and *Phyllanthus amarus,* and EDTA-drug control on lead-intoxicated rats

tannins, saponins and glycosides. Previous studies have confirmed and attributed these to the bitter taste and medicinal properties of *Vernonia amygdalina* [32,33] and to the phytotherapeutic abilities of *Phyllanthus amarus* [34,21,20].

The kidneys excrete a variety of waste products produced by metabolism into the urine which include the nitrogenous wastes urea, from protein catabolism, and uric acid, from nucleic acid metabolism [35]. Increased levels of serum urea and creatinine were exhibited in the lead-intoxicated control, suggesting the inability of the kidneys to excrete these products. This dysfunction could arise as a result of increased glomerular filteration pressures, reduced tubule urea reabsorption and delayed response to fluid restriction [36]. The substantial increases in these kidney biomarkers as detected in the intoxicated control corroborate with the research findings of Suradkar et al. [37].

A major demerit of chelation therapy is the random and erratic removal of vital minerals. Other side effects of chelation therapy include dehydration, hypocalcemia, increased enzymes as would be detected in liver function test, allergic reactions [10].

Studies have shown that lead affects the sodium ion concentration, which is responsible for numerous vital biological activities like generation of action potentials in the excitatory tissues for the purpose of cell to cell communication, uptake of neurotransmitters (choline, dopamine and GABA) and regulation of uptake and retention of calcium by synaptosomes [38]. Similarly affected is potassium, the principal cation of intracellular fluids which is involved in membrane

function [39]. According to Singer and Brenner [40] the basolateral Na+, K+-ATPase pump actively transports K+ in and Na+ out of the cell in a 2:3 ratio, and the passive outward diffusion of K+ is quantitatively the most important factor that generates the resting membrane potential; its chronic depletion may cause metabolic alkalosis by increasing urinary acid excretion. The intoxicated control revealed remarkably decreased level of serum sodium and potassium ions, indicating a substantial deterioration of these vital biochemical progressions. Treatment groups of the DTC, *Vernonia amygdalina* and *Phyllanthus amarus* significantly reversed these outcomes.

Thus, it could be deduced that within the same treatment period of the EDTA chelator, the aqueous extracts of *Vernonia amygdalina* and *Phyllanthus amarus* truncated the serious impairment of the normal functioning the aforementioned sodium-dependent processes accrued to the interaction between lead and sodium.

Furthermore, the histopathological evaluation in this study further buttressed the probable amelioration of kidney damage in the experimental rats by the aqueous plant extracts. Stromal proliferation with congestion, compressed tubules, shrunken glomeruli were characteristic of the intoxicated control. In contrast, the observed slightly enlarged tubules, oedemation and slightly shrunken glomeruli in the treated groups, especially those treated with *P. amarus* in which stromal cells appeared normal; indicate possible reversal of kidney damage. The possible reason for the attenuation of tissue damage could be due to flavonoids and other phytoconstituents in the plants which have antioxidant effects, hence scavenging ROS and reducing lipid peroxidation of its membrane as well.

5. CONCLUSION

Reparation of significantly altered kidney function parameters, normalisation of important electrolyte imbalance and near restoration of nephritic tissues, have demonstrated that aqueous *Vernonia amygdalina* and *Phyllanthus amarus* can compete favourably with an EDTA chelation therapy in lead toxicity management. Hence, their alternative use as natural chelators should be encouraged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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