

CHELATION POTENTIAL OF AQUEOUS LEAF EXTRACTS OF *VERNONIA AMYGDALINA* AND *PHYLLANTHUS AMARUS* ON KIDNEY FUNCTIONS IN LEAD-INTOXICATED ALBINO WISTAR RATS

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 75g were divided into seven groups of six animals each: normal NC (normal saline) and intoxicated control IC (1000ppm lead acetate in water) for three weeks; Drug treated control DTC (75mg/kg Na₂EDTA co-administered with 5mg/kg Calcium); *V. amygdalina*-treated IT1 (100mg/kg) and IT2 (200mg/kg); *P. amarus*-treated IT3 (100mg/kg) and IT4 (200mg/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 (4.28 ± 1.38 mg/dL) levels of the intoxicated control IC. The DTC (10.16 ± 1.46 mmol/L), 100mg/kg VA (12.69 ± 1.59 mmol/L), 200mg/kg VA (10.48 ± 1.12 mmol/L), 100mg/kg PA (9.96 ± 1.83 mmol/L) and 200mg/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), 100mg/kg VA (2.22 ± 0.43 mg/dL), 200mg/kg VA (2.07 ± 0.416 mg/dL), 100mg/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.

Key words: 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

45 cognitive development and intellectual performance in children and to increase blood
46 pressure and cardiovascular diseases incidence in adults [2]. Lead poisoning typically results
47 from ingestion of food or water contaminated with lead, but may also occur after accidental
48 ingestion of contaminated soil, dust, or lead-based paint [3]. A series of lead poisonings in
49 Zamfara State, a poor state blessed with abundant mineral resources, Nigeria, led to the
50 deaths of at least 163 people between March and June 2010 [4]. The lead poisoning
51 remained the cause of high mortality of children which was initially attributed to malaria [4].
52 Many heavy metals, including lead are known to induce over-production of reactive oxygen
53 species (ROS), which are the by-products of many degenerative reactions in many tissues,
54 hence will affect the regular metabolism by damaging the cellular components [5]. In addition,
55 ROS are highly reactive to membrane lipids, protein and DNA. They are believed to be the
56 major contributing factors to stress injuries and to cause rapid cellular damage [6]. Oxidative
57 stress has been reported as a major mechanism of lead induced toxicity. Under the influence
58 of lead, onset of oxidative stress occurs on account of two pathways which operate
59 simultaneously: first, the generation of ROS, like hydroperoxides, singlet oxygen and
60 hydrogen peroxide; and second, the depletion of antioxidant reserves [7]. Lead exposure has
61 also been shown to exhibit enhanced rate of liver lipid peroxidation and alter antioxidant
62 defense systems in rats [8].

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

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

105 resulting powder weight of 100g each was soaked in 2000ml of distilled water for 24 hours.
106 They were both filtered (Whatman No.1 paper) and the filtrates concentrated by evaporation
107 at 50°C in a water bath with further dryness in a Gallenkamp oven at 80°C until constant
108 weights were obtained.

109 **2.3 Phytochemical screening**

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

114 **2.4 Laboratory animals**

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

119 **2.5 Induction of lead intoxication**

120 1000ppm of lead acetate in distilled water was given orally as drinking water to the
121 intoxicated groups continuously for three (3) weeks, as modified [31].

122 **2.6 Experimental design**

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

- 126 • Non-intoxicated control received normal saline
- 127 • Intoxicated control received no treatment but normal saline
- 128 • EDTA-Drug treated control received 75mg/kg b.w Na₂EDTA + 5mg/kg Calcium
- 129 • Intoxicated treated IT 1 received 100mg/kg *V. amygdalina*
- 130 • Intoxicated treated IT 2 received 200mg/kg *V. amygdalina*
- 131 • Intoxicated treated IT 3 received 100mg/kg *P. amarus*
- 132 • Intoxicated treated IT 4 received 200mg/kg *P. amarus*

133 **2.7 EDTA Drug-treatment**

134 75mg/kg/b.w Disodium EDTA (BDH, UK) was dissolved, co-administered with 5mg/kg/b.w
135 Calcium (as Calcium lactate pentahydrate, Meyer Organics, PVT Ltd, India) in normal saline
136 and given orally by gavage.

137 **2.8 Collection and analysis of samples**

138 At the end of the experimental period, the animals were anaesthetized with dichloromethane
139 and sacrificed, with ethical consent from the Center of Biochemistry and Scientific research
140 unit. Whole blood was collected by cardiac puncture and was centrifuged after clotting at
141 5000 rpm for ten (10) minutes to obtain serum. Urea and creatinine concentrations were
142 analyzed using Diacetylmonoxime method [28] and the Jaffe's reaction method by Seaton
143 and Ali [29] respectively. Electrolytes were estimated with an automated ion selective
144 electrode analyzer (BAUR-biloyte Spin 5, PCE-Zion). All chemicals and reagents used were
145 of standard analytical grade.

146 **2.9 Statistical analysis**

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

150 **2.10 Histological assessment**

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

153 **3. RESULTS AND DISCUSSION**

154 **3.1 Phytochemical analysis**

155 The percentage yields of *Vernonia amygdalina* and *Phyllanthus amarus* were 18.60% and
156 10.33% respectively. Phytochemical screening revealed the presence of alkaloids,
157 flavonoids, saponins, tannins and cyanogenic glycosides as shown in 'Table 1'.
158

159 **Table 1: Qualitative phytochemical analysis of the plant samples**

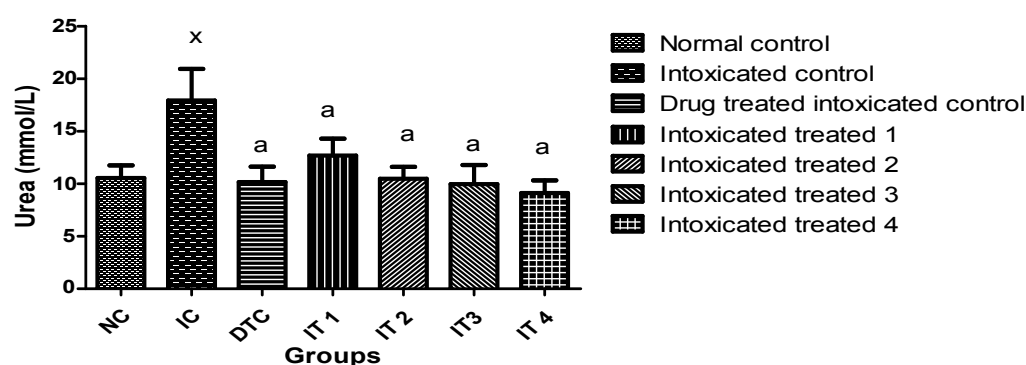
Phytochemical	<i>V. amygdalina</i>	<i>P. amarus</i>
Saponins	+	+
Alkaloids	+	+

Flavonoids	+	+
Tannins	+	+
Cyanogenic glycosides	+	+

+ Indicates presence of compounds

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 'Figure 1'. The drug-treated control DTC (10.16±1.46), IT1 [100mg/kg VA] (12.69±1.59), IT2 [200mg/kg VA] (10.48±1.12), IT3 [100mg/kg PA] (9.96±1.83) and IT4 [200mg/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).**



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

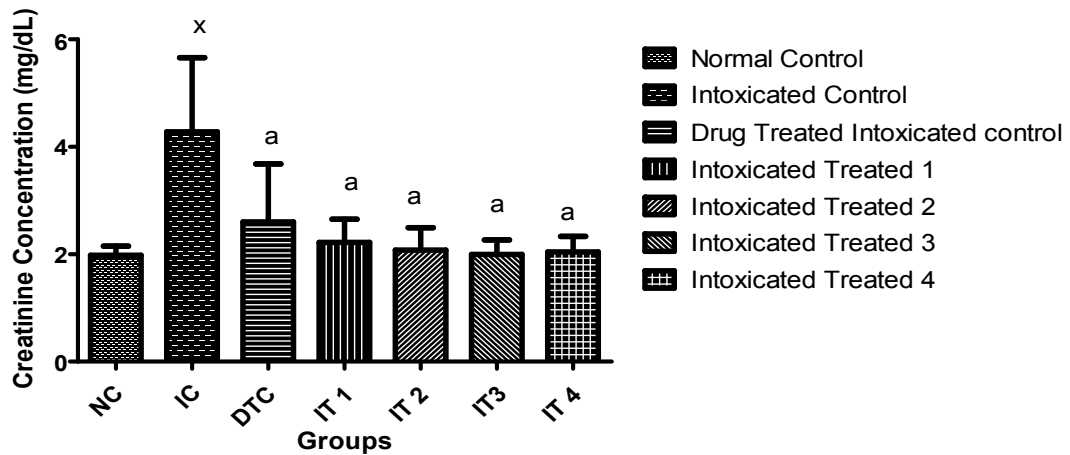
Fig. 1. Comparative effects of graded doses of aqueous *Vernonia amygdalina* and *Phyllanthus amarus*, and EDTA-drug control on serum urea concentration of lead-intoxicated rats

3.3 Effects of aqueous *Vernonia amygdalina* and *Phyllanthus amarus* on serum creatinine concentrations of lead-intoxicated rats

'Figure 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 [100mg/kg VA] (2.22±0.43), IT2 [200mg/kg VA] (2.07±0.416), IT3 [100mg/kg PA] (1.99±0.27) and IT4

182 [200mg/kg PA] (2.04 ± 0.28) exhibited a significant reversal ($p < 0.05$) of the observed
 183 significant increase ($p < 0.05$) of serum creatinine levels in the intoxicated control IC
 184 (4.28 ± 1.38). The treatment groups showed no significant variation to the normal control
 185 (1.97 ± 0.17).

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

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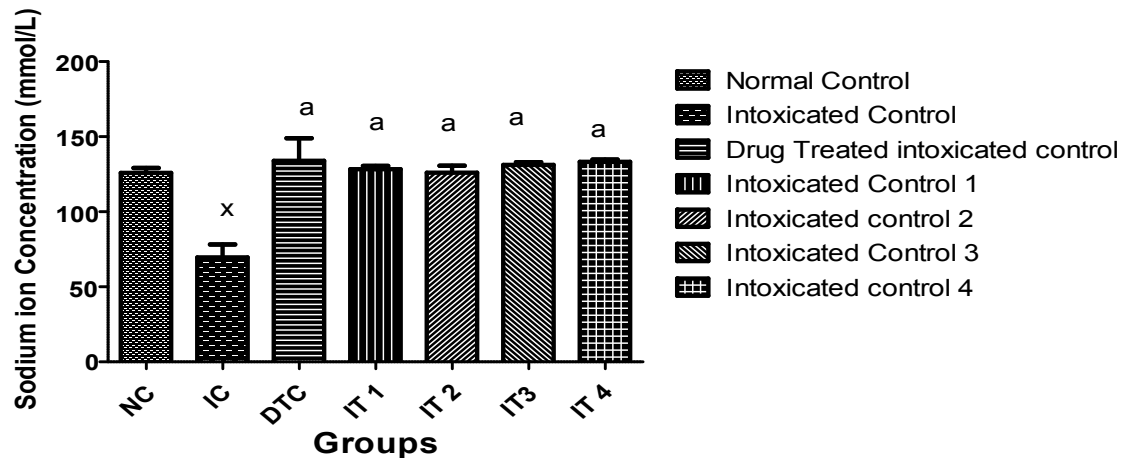
Fig. 2. Comparative effects of graded doses of aqueous *Vernonia amygdalina* and *Phyllanthus amarus*, and EDTA-drug control on serum creatinine concentration of lead-intoxicated rats

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3.4 Effects of aqueous *Vernonia amygdalina* and *Phyllanthus amarus* on serum sodium ion concentration of lead-intoxicated rats

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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 'Figure 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 [100mg/kg VA] (128.30 ± 2.16), IT2 [200mg/kg VA] (126.0 ± 4.60), IT3 [100mg/kg PA] (131.20 ± 1.83) and IT4 [200mg/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.

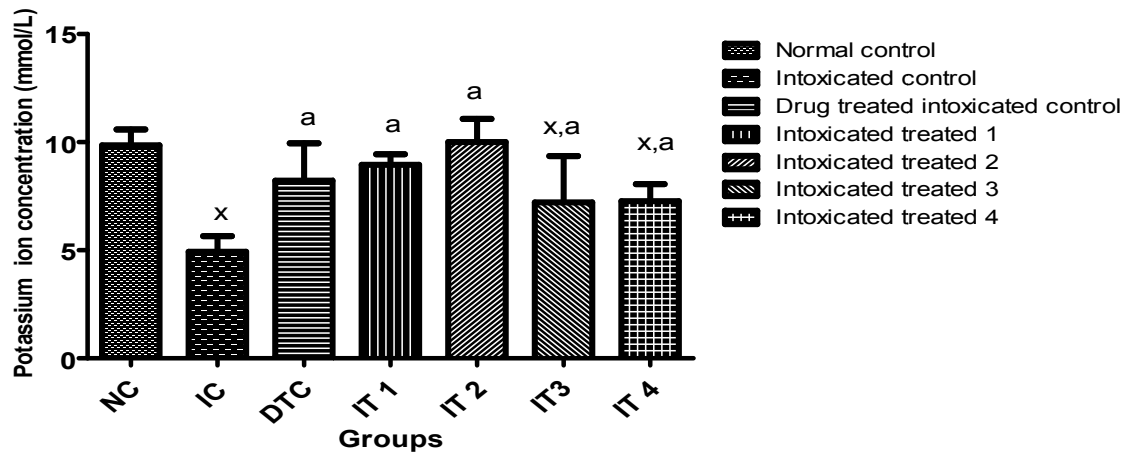


Comparison of serum sodium ion levels of different experimental groups.
Values are mean $\pm$ SD, n = 6, P < 0.005.
x vs NC and a vs IC.

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

3.5 Effects of aqueous *Vernonia amygdalina* and *Phyllanthus amarus* on serum potassium ion concentration of lead-intoxicated rats

'Figure 4' shows the effects of graded doses of *V. amygdalina* and *P. amarus* on serum potassium ion concentration of intoxicated rats. The intoxicated control IC (4.93 $\pm$ 0.72) exhibited a significant decline in serum potassium levels compared to the normal control (9.85 $\pm$ 0.74). The DTC (8.22 $\pm$ 1.73), IT1 [100mg/kg VA] (8.95 $\pm$ 0.49), IT2 [200mg/kg VA] (10.0 $\pm$ 1.07), IT3 [100mg/kg PA] (7.21 $\pm$ 2.14) and IT4 [200mg/kg PA] (7.27 $\pm$ 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.



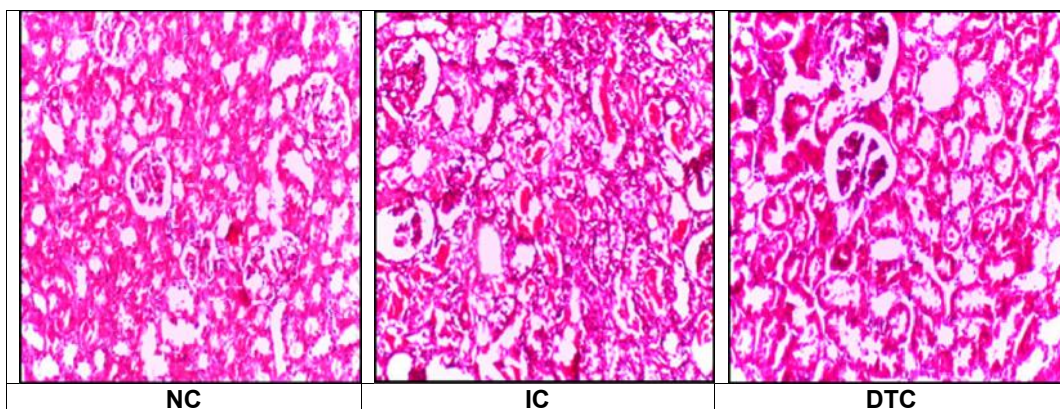
comparison of serum potassium levels of different experimental groups. values are mean $\pm$ SD, n=6, P < 0.05.
x vs NC and a vs IC

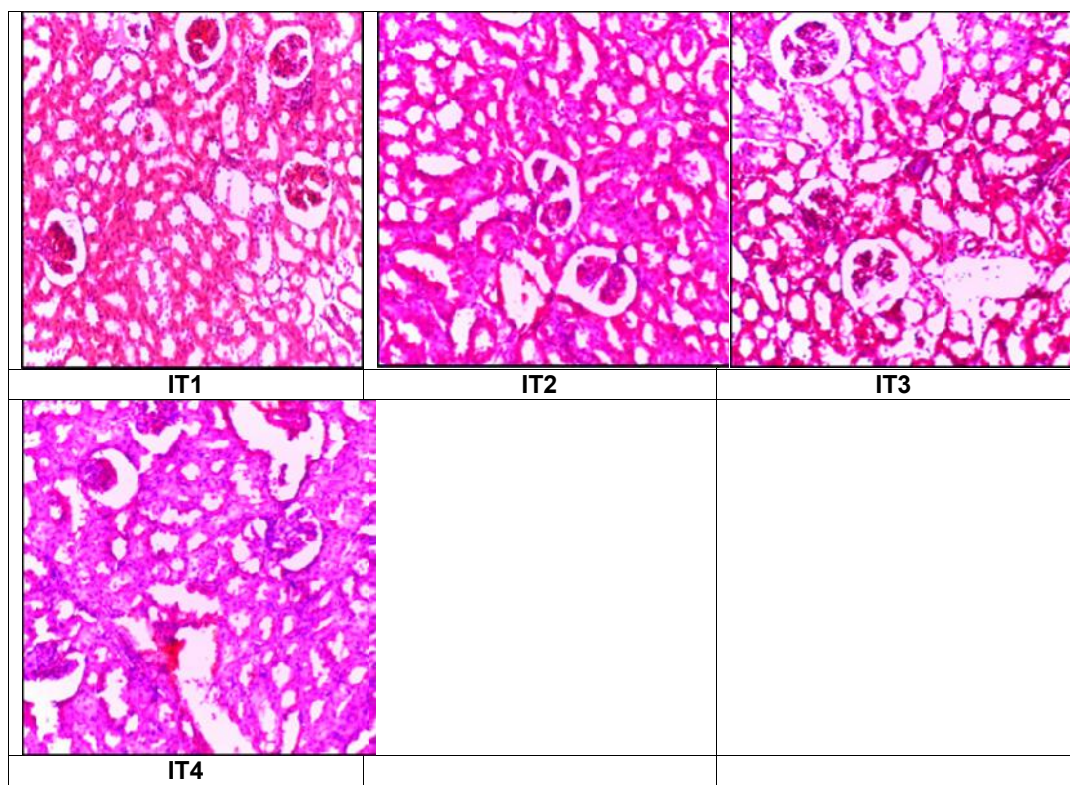
Fig. 4. Comparative effects of graded doses of aqueous *Vernonia amygdalina* and *Phyllanthus amarus*, and EDTA-drug control on serum potassium ion concentration of lead-intoxicated rats

3.6 Histological assessment results

In the intoxicated control, stromal proliferation with congestion, compressed tubules, shrunken glomeruli with some cystically 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 'Table 2'.

Table 2. Comparative kidney histopathological assessments of graded doses of aqueous *Vernonia amygdalina* and *Phyllanthus amarus*, and EDTA-drug control on lead-intoxicated rats





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239 DISCUSSION

240 Research evidence has shown that lead is a toxic agent with multiple target organs such as
 241 the hematopoietic system, immune system, kidneys and nervous system [30, 31].

242 Administration of various antioxidants can prevent or subdue various toxic effects of lead and
 243 generation of oxidative stress [12]. Results of phytochemical analysis of both plants indicated
 244 the presence of alkaloids, flavonoids, tannins, saponins and glycosides. Previous studies
 245 have confirmed and attributed these to the bitter taste and medicinal properties of *Vernonia*
 246 *amygdalina* [32, 33] and to the phytotherapeutic abilities of *Phyllanthus amarus* [34, 21, 20].

247 The kidneys excrete a variety of waste products produced by metabolism into the urine which
 248 include the nitrogenous wastes urea, from protein catabolism, and uric acid, from nucleic acid
 249 metabolism [35]. Increased levels of serum urea and creatinine were exhibited in the lead-
 250 intoxicated control, suggesting the inability of the kidneys to excrete these products. This
 251 dysfunction could arise as a result of increased glomerular filtration pressures, reduced
 252 tubule urea reabsorption and delayed response to fluid restriction [40]. The substantial

253 increases in these kidney biomarkers as detected in the intoxicated control corroborate with
254 the research findings of Suradkar et.al [36].

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

258 Studies have shown that lead affects the sodium ion concentration, which is responsible for
259 numerous vital biological activities like generation of action potentials in the excitatory tissues
260 for the purpose of cell to cell communication, uptake of neurotransmitters (choline, dopamine
261 and GABA) and regulation of uptake and retention of calcium by synaptosomes [37]. Similarly
262 affected is potassium, the principal cation of intracellular fluids which is involved in membrane
263 function [38]. According to Singer and Brenner [39] the basolateral Na⁺, K⁺-ATPase pump
264 actively transports K⁺ in and Na⁺ out of the cell in a 2:3 ratio, and the passive outward
265 diffusion of K⁺ is quantitatively the most important factor that generates the resting
266 membrane potential; its chronic depletion may cause metabolic alkalosis by increasing
267 urinary acid excretion. The intoxicated control revealed remarkably decreased level of serum
268 sodium and potassium ions, indicating a substantial deterioration of these vital biochemical
269 progressions. Treatment groups of the DTC, *Vernonia amygdalina* and *Phyllanthus amarus*
270 significantly reversed these outcomes.

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

275 **Furthermore, the histopathological evaluation in this study further buttressed the probable**
276 **amelioration of kidney damage in the experimental rats by the aqueous plant extracts.**
277 **Stromal proliferation with congestion, compressed tubules, shrunken glomeruli were**
278 **characteristic of the intoxicated control. In contrast, the observed slightly enlarged tubules,**
279 **oedemation and slightly shrunken glomeruli in the treated groups, especially those treated**
280 **with *P. amarus* in which stromal cells appeared normal; indicate possible reversal of kidney**
281 **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.

4. 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.

REFERENCES

- [1] Pitot CH, Dragan PY. Chemical carcinogenesis. Casarett and Doull's toxicology. 5th ed. New York: McGraw Hill; 1996.
- [2] Commission of the European Communities (CEC). Setting maximum levels for certain contaminants in foodstuffs. Brussels: Official Journal of the European Communities. Commission regulation (EC) No. 221/2002 of 6 February 2002 amending regulation (EC) NO. 466/2002; 2002.
- [3] Bergeson LL. The proposed lead NAAQS: Is consideration of cost in the clean air act's future? Environmental Quality Management. 2008; 18:79.
- [4] Yahaya S. Lead poisoning from mining kills 163 in Nigeria. Reuters. Thomson Reuters. Accessed 4 June 2010. Available: <http://www.theworld.org/2010/06/09>
- [5] Foyer CH, Noctor G. Oxygen processing in photosynthesis: regulation and signaling. New Phytol. 2002; 146:359–388.
- [6] Aly AA, El-Beltagi HES. Influence of ionizing irradiation on the antioxidant enzymes of *Vicia faba*. L. Grasa Y Aceite. 2010; 61(3):288–294.
- [7] Flora SJS. Nutritional components modify metal absorption, toxic response and chelation therapy. J Nut Environ Med. 2002; 12:53–67.

- 308 [8] Adegbesan BO, Adenuga GA. Effect of lead exposure on liver lipid peroxidative and
309 antioxidant defense systems of protein-undernourished rats. Biol Trace Elem Res. 2007; 116:
310 219-25.
- 311 [9] Andersen O. Principles and recent developments in chelation treatment of metal
312 intoxication. Chem. Rev. 1999; 99:2683–2710.
- 313 [10] Kosnett MJ. Chelation for Heavy Metals (Arsenic, lead and mercury): Protective or
314 perilous? Clinical Pharmacology and Therapeutics. 2010; 88(3):412-415.
- 315 [11] Chisolm JJ. Safety and efficacy of meso-2,3-dimercaptosuccinic acid (DMSA) in
316 children with elevated blood lead concentrations. Journal of Toxicology: Clinical Toxicology.
317 2000; 38 (4):365–75.
- 318 [12] Flora SJS, Pande M. Mehta A. Beneficial effect of combined administration of some
319 naturally occurring antioxidants (vitamins) and thiol chelators in the treatment of chronic lead
320 intoxication. Chem Biol Interact. 2003; 145:267-80.
- 321 [13] Mathews HB, Lucier GW, Fisher KD. Medicinal herbs in the United States: Research
322 needs. Environ. Health Perspect. 1999; 107(10):773-778.
- 323 [14] Bonsi, MLK, Oseji PO, Teach AK, Umunna MN. () Vernonia amygdalina as a
324 supplement of teff straw (Eragrostic tef) fed to Ethiopian sheep. Agro forestry systems. 1995;
325 31(3):229-244.
- 326 [15] Ijeh II, Ejike CECC. Current perspectives on the medicinal potential of Vernonia
327 amygdalina Del. J Med Plant Res. 2011; 5(7):1051–1061.
- 328 [16] Ibrahim NDG, Abdurahman EM, Ibrahim G. Elemental analysis of the leaves of
329 Vernonia amygdalina and its biological evaluation in rats. Niger. J. Natl Prod. Med. 2001;
330 5:13-16.
- 331 [17] Erasto P, Grierson DS, Afolayan AJ. Evaluation of Antioxidant activity and the fatty
332 acid profile of the leaves of Vernonia amygdalina growing in South Africa. Food Chemistry.
333 2007; 104:636–642

- 334 [18] Izevbigie, EB. Discovery of water soluble anticancer agents (edotides) from a
335 vegetable found in Benin city, Nigeria. *Exp. Biol.Med* 2003; 228(3):293-298.
- 336 [19] Ezirim AU, Okochi VI, James AB, Adebeshi OA, Ogunnowo S, Odeghe OB. Induction
337 of apoptosis in myelogenous leukemic K562 cells by ethanolic leaf extract of *Annona*
338 *muricata*. *IJDAD*. 2013; 2,2.
- 339 [20] Patel JM, Tripathi P, Sharma V, Nagendra S.) *Phyllanthus amarus*: ethnomedicinal
340 uses, phytochemistry and pharmacology. A review *Journal of ethnopharmacology*. 2011;
341 138(2);286-313.
- 342 [21] Odetola AA, Akojenu SM. Anti diarrhea and gastrointestinal potential of the aqueous
343 extract of *Phyllanthus amarus*. *Afr J Med Sci*. 2000; 7(2):119-22.
- 344 [22] Moshi MJ, Uiso FC, Mahunnah RLA, Malele SR, Swai ABM. A study of the effect of
345 *Phyllanthus amarus* extracts on blood glucose in rabbits. *Int. J. Pharmacog*. 1997; 35:167-
346 173.
- 347 [23] Kumar KBH, Kuttan R. Chemoprotective activity of an extract of *Phyllanthus amarus*
348 against cyclophosphamide induced toxicity in mice. *Phytomed*. 2005; 12(6):494-500
- 349 [24] Huang RL, Wang MX, Thyagarajan SP. Screening of 25 compounds isolated from
350 *Phyllanthus* species for anti-human hepatitis B virus in vitro. *Phytother. Res*. 2003; 17:449-
351 453.
- 352 [25] Santos ARS, Campos ROP, Miguel OG, Filho VC, Siani AC, Yunes RA, et al.
353 Antinociceptive properties of extracts of new species of plants of the genus *Phyllanthus*
354 (*Euphorbiaceae*). *J. Ethnopharmacol*. 2000; 72:229-238.
- 355 [26] Brown MJ, Wills T, Omalu B, Leiker R. Death resulting from hypocalcemia after
356 administration of edentate disodium. *Pediatrics*. 2006; 118(2):534-6.
- 357 [27] Trease G, Evans WC. *A Textbook of Pharmacognosy*. 13th ed. London: W.B.
358 Saunders; 1989.

359 [28] Wybenga DR, DiGiorgio J, Pileggi VJ. Manual and automated methods for urea
360 nitrogen measurement in whole serum. Clin Chem. 1971; 17:891-895.

361 [29] Seaton B, Ali A. Simplified manual high performance clinical chemistry methods for
362 developing countries. Med Lab Sci. 1984; 41(4):327-336.

363 [30] Kalia K, Flora SJS. Strategies for Safe and Effective Treatment for Chronic Arsenic
364 and Lead Poisoning. J Occup Health. 2007; 47:1-21.

365 [31] Velaga, MK, Basuri, CK, Taylor KSR, Yallapragada PR, Rajanna S, Rajanna B.
366 Ameliorative effects of Bacopa monniera on lead-induced oxidative stress in different regions
367 of rat brain. Drug and Chemical Toxicology. 2014; 37(3):357-364.

368 [32] Nangendo G, Stein A, Gelens M, de Gier A, Albricht R. Quantifying differences in
369 biodiversity between a tropical forest area and a grassland area subject to traditional burning.
370 For. Ecol. Manage. 2002; 164:109-120.

371 [33] Favi, F, Cantrell CI, Mebrahtu T, Kraemer ME. Leaf peltate glandular trichomes of
372 Vernonia galamensis ssp. Galamensis var. ethiopica gilbert: Development, ultrastructure, and
373 chemical composition. Int. J. Plant Sci. 2008; 169:605-614.

374 [34] Reichert R. Phytotherapeutic alternative of hepatitis. Rev National Med. 1997; 103-
375 108.

376 [35] Overu SS, Berepubo N.A, Nobu MB. Biochemical blood parameters in semi-adult
377 rabbit experimentally fed crude oil contaminated diets. Afri.J.biotechnol. 2004; 3(6):343-345

378 [36] Suradkar SG, Ghodasara DJ, Vihol P, Patel J, Jaiswal V, Prajapati KS. Haemato-
379 biochemical alterations induced by lead-acetate toxicity in wistar rats. Vet world. 2009;
380 2(11):429-431.

381 [37] Bressler, J, Kim KA, Chakraborti T, Goldstein G. Molecular mechanisms of lead
382 neurotoxicity. Neurochem Res. 1999; 24:595-600.

383 [38] Bender DA, Mayes PA. Vitamins and Minerals. In: Murray RK, Granner DK, Mayes
384 PA, Rodwell VW, editors. Harper's illustrated biochemistry. 26th ed. New york: McGraw-Hill.
385 2003.

386 [39] Singer GG, Brenner BM. Fluid and electrolyte disturbances. In: Kasper DL,
387 Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, editors. Harrison's principle of
388 internal medicine. 16th ed. Chicago: McGraw-Hill. 2005.

389 [40] Denker BM, Brenner BM. Azotemia and urinary abnormalities. In: Kasper DL,
390 Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, editors. Harrison's principle of
391 internal medicine. 16th ed. Chicago: McGraw-Hill. 2005.

392

393