

Nephroprotective Effects of Aqueous Extracts of *Hibiscus sabdariffa* Calyces and *Jatropha curcas* Leaves against Lead Toxicity

Okonkwo, Chioma Joy*, Belonwu Donatus Chuka, Monago-Ighorodje C. Comfort,
Okonkwo Chinedu Joseph, Oforibo, Esther Clement

Department of Biochemistry, University of Port Harcourt, Rivers state, Nigeria

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Abstract Background: Lead is a persistent heavy metal which has been found to be a multi-organ toxicant. In recent times, plants containing natural compounds with both chelating and antioxidant activities are considered as promising candidates for managing lead toxicity. **Objectives:** The present investigation was undertaken to evaluate the ameliorative potentials of aqueous extracts of *Jatropha curcas* (JC) leaves and *Hibiscus sabdariffa* (HS) calyces against renal toxicity resulting from lead acetate exposure. **Materials and Methods:** Ten groups of male Wistar rats were taken: The first and second groups represented the normal and lead acetate (PbA) (50mg/kg b.w) control groups respectively while groups 3 and 4 were given 100mg/kg JC and 200 mg/kg JC respectively. Groups 5 and 6 received 250 mg/kg HS and 500 mg/kg HS respectively. Groups 7-10 were given PbA (50mg/kg) and 100mg/kg JC, 200 mg/kg JC, 250mg/kg HS, 500mg/kg HS respectively. Rats were orally administered their relevant doses for 28 days. Blood samples were collected from heart puncture at the end of the experiment (28 days) for renal function analysis. **Result:** Serum creatinine and urea concentrations were significantly higher ($p < 0.05$) in the lead acetate group compared to the normal control. The renal morphology of the lead-exposed rats also revealed the degeneration and necrosis in tubules and swelling in the glomerulus. The significant increase in renal markers and morphological alterations after lead toxicity induction were moderately normalized by the administration of aqueous extracts of *Jatropha curcas* and *Hibiscus sabdariffa*. **Conclusions:** Results demonstrated moderate beneficial effects of *J. curcas* and *H. sabdariffa* treatment in managing Pb-induced tissue damage in the kidney.

Keywords Lead Toxicity, Nephroprotection, *Jatropha curcas*, *Hibiscus sabdariffa*

1. Introduction

As a toxin, lead induces a wide range of physiological, biochemical and behavioural dysfunction; in particular, the kidney, liver, spleen, testes have been identified as key target organs for lead toxicity [1-3]. Lead toxicity occurs when lead accumulates in the body over a period of months or years. Elevated blood lead levels of 0.3 mg/ml and above have been found to cause toxic effects in humans and animals [4]. There is growing concern that a significant number of people especially children remain at risk from lead exposure in different parts of the world. Common environmental lead exposure sources to the general population include leaded water supply pipes, lead based paints and ceramics, gasoline, contaminated food, drinks, cosmetics and medicine [5].

The kidney plays a principal role in the excretion of metabolic wastes and in the regulation of intracellular fluid volume, electrolyte composition, and acid-base balance [6]. A toxic insult to the kidney, therefore, could disrupt any or all of these functions and could have a profound effect on total body metabolism. Tiwari et al [7] demonstrated that lead was nephrotoxic even at low doses. Renal tissue damage such as tubular atrophy, inflammation and necrosis have also been observed in lead treated mice. Generally, most metals including lead are concentrated by the kidney and this tends to produce a spectrum of biochemical injuries to the nephron. The extent of lead-induced nephrotoxicity is determined by factors such as the presence of high-affinity metal-binding proteins, cell type, exposure frequency and dietary status [8].

Consequently, there is now resurged interest in seeking

strategies to reduce the toxicity associated with lead exposure. Several plants have been screened for their protective efficacy against lead toxicity in animal model [9-12]. Plants with appreciable levels of antioxidant and chelating activities are considered as promising candidates for mitigating lead toxicity [13]. *Jatropha curcas* and *Hibiscus sabdariffa* are two Nigerian plants with well-established nutraceutical properties. Asuk et al. [14] reported that *J. curcas* leaves were rich sources of both macro and micro-nutrients. They observed that the polyphenol contents of the *J. curcas* leaves were high. Polyphenols are phytochemicals that exercise anti-oxidative activities [15]. *H. sabdariffa* is a medicinal and food plant rich in phytochemical compounds which are the source of its biological properties [16]. Although from literature, both plants have been reported to have demonstrated significant antioxidant activities, there is limited information on their efficacy in lead-induced oxidative stress in the kidney of rats. Hence the present investigation was designed to study the protective efficacy of aqueous leaf extracts of *J. curcas* and *H. sabdariffa* respectively in the kidney upon exposure to lead.

2. Materials and Methods

2.1. Chemicals

Lead acetate $Pb(CH_3CO_2)_2$ and all other chemicals used in the study were obtained from Sigma-Aldrich Corporation (St. Louis, Missouri, USA).

2.2. Plant material

The leaves of *J. curcas* were obtained from Abuja park of the University of Port Harcourt, Rivers state, Nigeria while the *H. sabdariffa* calyces were obtained from a local market in Obio/Akpor Local Government Area of Rivers State, Southern Nigeria. The plant material was washed using double distilled water and then dried at room temperature for 48 to 92 h and then grounded into powder and stored at room temperature until use.

2.3. Animals care handling and Experiment Design

A total of 60 male adult albino rats of varying weights (90 – 200g), respectively bred in the animal house of the Department of Physiology, University of Port Harcourt was obtained for this study. The rats were divided into ten (10) groups and housed in polypropylene cages were maintained under standard conditions prescribed by the committee for control and supervision on experiments on animals (CPCSEA). Before the initiation of the experiment, the rats were left to acclimatize for a period of seven (7) days to natural environmental conditions of 12 hours light and 12 hours dark cycle with an ambient temperature of (25

± 2) °C. During the period of acclimatization and through the experiment the rats were fed with rat feed pellets and clean drinking water *ad libitum*. The research lasted for 28 days.

Group 1: water and rat feed only (normal control)

Group 2: 50 mg/kg PbA (toxic control)

Groups 3 & 4: 100 and 200 -mg/kg *J. curcas* extracts respectively

Groups 5 & 6: 250 and 500 -mg/kg *H. sabdariffa* extracts respectively

Groups 7 & 8: PbA (50mg/kg) + 100 and 200 -mg/kg *J. curcas* extracts respectively

Groups 9 & 10: PbA (50mg/kg) + 250 and 500 -mg/kg *H. sabdariffa* extracts respectively

At the end of the experiment, the animals were fasted overnight. The blood samples were collected, some of which was centrifuged to obtain the serum which was kept frozen at -20 °C until used for biochemical analysis of electrolytes, urea and creatinine. Pieces of tissues from kidney were immediately kept in 10% of formalin fixative to study histological alterations.

2.4. Biochemical Analysis

2.4.1. Serum Biochemical Parameters

Serum urea and creatinine concentration were estimated using an automatic clinical chemistry analyzer (Mindray BS800, Shenzhen, China) while the serum electrolytes were determined using ISO 4000 Automated electrolyte analyzer. SFRI, France.

2.4.2. Histological Examinations

For light microscopy examination, the formalin-fixed tissues were dehydrated through ascending grades of alcohol, cleared in three changes of xylene, and were embedded in paraffin. Serial sections, each of 4-micron thickness, were cut and stained with H and E as per standard protocols [17].

2.4.3. Statistical Analysis

The data were subjected to statistical analysis by using statistical package for social sciences (SPSS) version. Differences between means were tested using Student t-tests and significance was set at $P < 0.05$ and the results are expressed as Mean \pm Standard Error of Mean (SEM).

3. Results

3.1. Biochemical Parameters

The results as presented in Table 1 showed that the serum urea and creatinine increased significantly ($p < 0.05$) in the lead acetate exposed group compared to the control group. On the other hand, there was no significant difference for the serum electrolytes levels in both control

and experimental groups (Table 2). Our results further showed significant decreases ($p < 0.05$) in the serum creatinine and urea in the groups that received

concomitant treatment of lead acetate and 100 mg/kg JC, 200 mg/kg JC and 500 mg/kg HS extracts respectively compared to the lead acetate group.

Table 1. Changes in serum urea and creatinine of control and experimental groups

GROUP	UREA (mmol/L)	CREATININE (mmol/L)
Normal Control	7.50 ± 0.90b	0.27 ± 0.06b
Lead acetate (PbA) control	11.70 ± 1.30a	0.52 ± 0.11a
100mg JC extract	6.16 ± 0.75b	0.30 ± 0.05b
200mg JC extract	8.80 ± 0.78	0.35 ± 0.07b
250mg HS extract	6.60 ± 0.58b	0.38 ± 0.05
500mg HS extract	8.10 ± 1.26	0.39 ± 0.07
PbA + 100mg JC extract	6.00 ± 0.84b	0.42 ± 0.06
PbA + 200mg JC extract	5.30 ± 0.46b	0.38 ± 0.04b
PbA + 250mg HS extract	8.06 ± 1.06	0.46 ± 0.08
PbA + 500mg HS extract	7.10 ± 0.84b	0.40 ± 0.05b

Values are mean ± SE of triplicate determinations. Superscript a and b indicates significant difference from normal and PbA control group respectively ($p < 0.05$) JC- *Jatropha curcas* HS- *Hibiscus sabdariffa*.

Table 2. Changes in serum electrolytes concentration (mmol/L) of control and experimental groups after 28 days of treatment

GROUP	Na ⁺	K ⁺	HCO ₃ ⁻	Cl ⁻
Normal Control	143.00 ± 5.67	11.17 ± 1.05	20.67 ± 2.17	110.67 ± 9.60
Lead acetate (PbA) control	145.00 ± 6.10	13.10 ± 1.20	23.67 ± 1.71	116.00 ± 10.05
100mg JC extract	143.30 ± 3.82	9.67 ± 1.00	22.50 ± 2.30	115.30 ± 8.10
200mg JC extract	145.70 ± 4.50	10.13 ± 1.08	20.67 ± 2.04	114.30 ± 7.50
250mg HS extract	145.30 ± 5.40	11.30 ± 1.14	19.33 ± 1.67	114.30 ± 6.78
500mg HS extract	142.00 ± 4.10	10.35 ± 1.07	21.33 ± 2.26	110.00 ± 9.58
PbA + 100mg JC extract	142.00 ± 3.80	9.97 ± 0.96	20.67 ± 2.07	111.50 ± 7.95
PbA + 200mg JC extract	145.30 ± 3.60	8.37 ± 0.87 ^{ab}	20.00 ± 1.98	114.33 ± 9.56
PbA + 250mg HS extract	142.60 ± 3.10	11.17 ± 1.02	22.33 ± 2.56	112.67 ± 7.57
PbA + 500mg HS extract	143.50 ± 4.02	10.45 ± 1.11	20.70 ± 2.38	113.50 ± 8.65

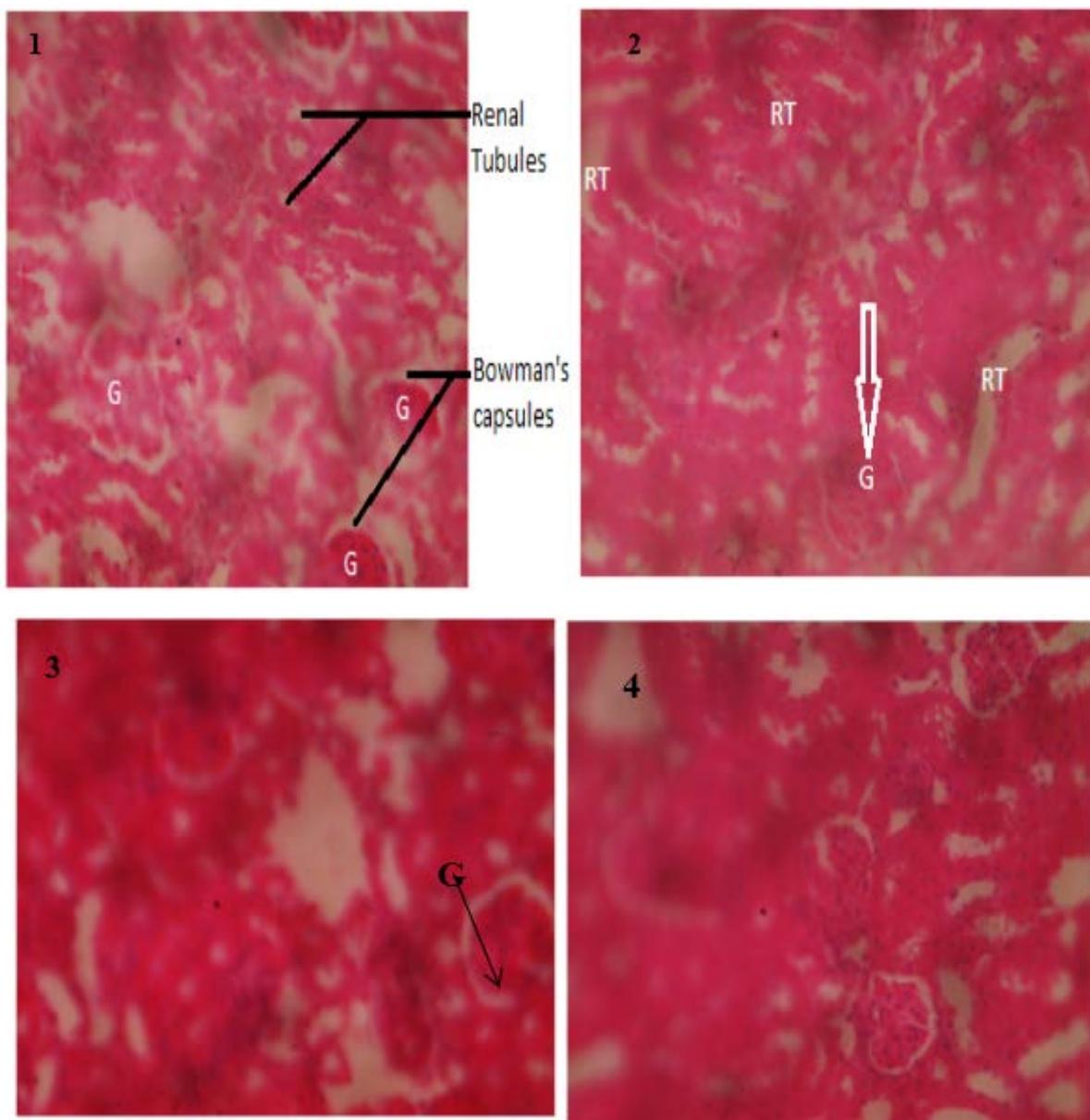
Values are mean ± SE of triplicate determinations. Superscript a and b indicates significant difference from normal and PbA control group respectively ($p < 0.05$) JC- *Jatropha curcas* HS- *Hibiscus sabdariffa*.

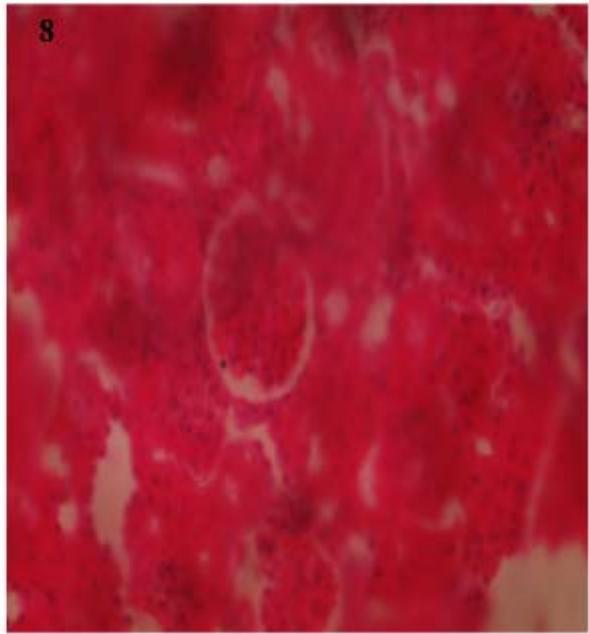
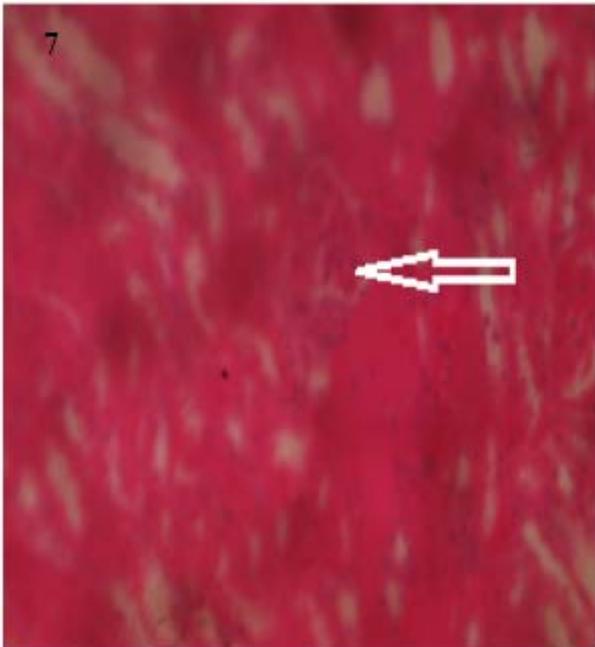
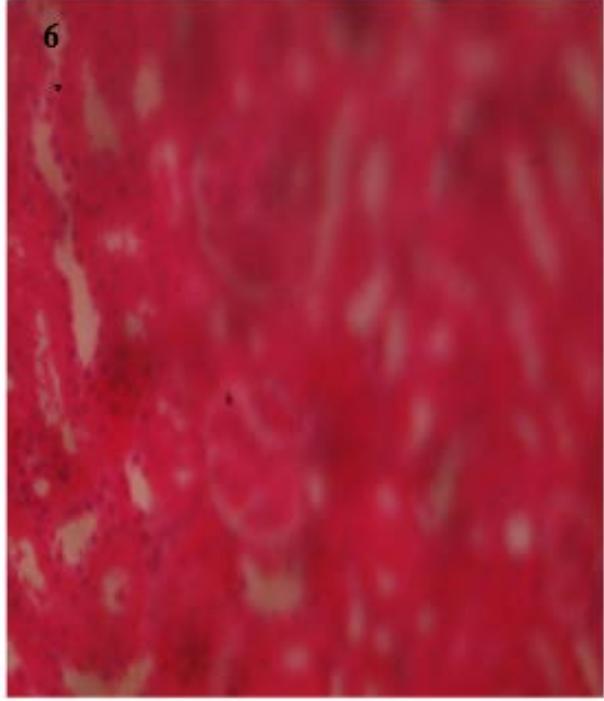
3.3. Histopathological Examination

The examination of the photomicrographic section of the kidney of the normal control rats (Figure 1) and the groups that received the plant extracts only (Figures 3-6) showed histological normal kidney architecture with renal tubules, glomeruli and Bowman's capsular space appearing normal. The histological sections of the PbA exposed rats revealed distorted kidney with glomerulus having obliterated Bowman's capsule (Figure 2). Concomitant treatment of PbA exposed rats with 200 mg/kg *J. curcas* extract for 28 days induced a moderate restoration of the kidney architecture (Figure 8). A similar trend was also observed for the PbA exposed rat group that received 500 mg/kg *H. sabdariffa* extract (Figure 10).

Photomicrographic kidney section from control rat

showing normal architecture (Figure 1, x400); PbA treated rats liver section showing obliterated Bowman's capsule (white arrow) (Figure 2, x400); 100 mg/kg *J. curcas* treated rat liver showing normal appearance of renal tubules and glomerulus, G (black arrow) (Figure 3 x400); Kidney sections of rats treated with 200 mg/kg *J. curcas* (Figure 4, x400), 250 mg/kg *H. sabdariffa* (Figure 5, x400), 500 mg/kg *H. sabdariffa* (Figure 6, x400) all showed normal appearance of the kidney. Kidney section of rat that received both PbA and 100 mg/kg *J. curcas* showed a minor degree of renal damage (Figure 7, x400). Kidney sections of the PbA exposed rats that received 200 mg/kg *J. curcas* (Figure 8, x400), 250 mg/kg *H. sabdariffa* (Figure 9, x400) and 500 mg/kg *H. sabdariffa* (Figure 10, x400) all showed moderately restored renal architecture.





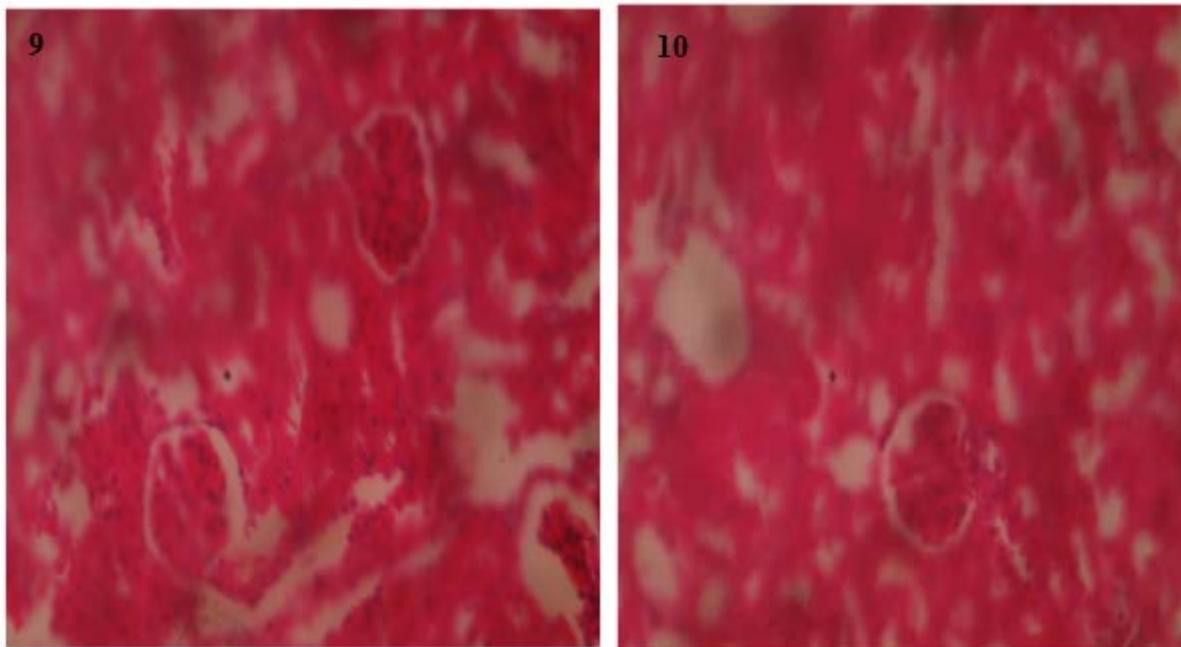


Figure 1-10. The histological examination of kidneys

4. Discussions

Lead is the most abundant of all the nephrotoxic elements, and there have been both extensive clinical studies of lead-induced nephropathy and corroborative studies in experimental animal models which have yielded extensive insight into both the mechanisms of cellular toxicity and factors regulating these processes [8, 18]. Oxidative damage has been identified as the main mechanism by which lead induces nephrotoxicity [19]. *J. curcas* and *H. sabdariffa* are two plants that have been used in traditional medicine to treat conditions such as anaemia, inflammation, and microbial activities. It has also been reported that both plants are sources of potent antioxidants [20-22]. The current investigation was designed to assess the nephroprotective potentials of *J. curcas* and *H. sabdariffa* against lead acetate-induced toxicity in the kidney. In this study, we observed that treatment with 50 mg/kg body weight of lead acetate for 28 days had a significant effect on serum renal markers and was associated with changes in kidney tissue including degeneration, vacuolization and debris.

In lead-induced nephrotoxicity, creatinine and blood urea levels become elevated in the blood. The biomarkers serum creatinine and blood urea are recommended for the assessment of kidney injury in preclinical studies as they are generally considered as more specific sensitive indicators of kidney damage. Low levels of creatinine and urea are normally found in the blood, but when the kidney is damaged or diseased, creatinine and urea accumulate in the bloodstream [23]. Asgharian et al. [24] reported a positive correlation between elevated blood lead levels and serum creatinine concentrations in animal models. Gennart

et al. [25] similarly found that serum creatinine and blood urea concentrations were elevated in lead-exposed subjects. In agreement with these findings, our results revealed that lead administration resulted in increased serum creatinine and blood urea concentration in the lead acetate exposed group compared to the normal group. Creatinine directly relates to the muscle mass, and its serum level is used to evaluate the glomerular filtration rate because it is readily filtered and not subjected to any significant tubular reabsorption [26]. The elevated creatinine levels observed in the PbA treated group suggests possible impaired renal function confirmed by inadequate clearance of creatinine by the kidney.

The kidney performs the major function of excreting drugs and metabolites from the system, regulation of extracellular pH, and electrolyte contents, hence, plays a central role in homeostasis [28]. In this study, concomitant administration of PbA and *J. curcas* resulted in decreased serum creatinine and urea concentrations in a dose-dependent manner. A similar trend was also observed in the experimental groups that received PbA and *H. sabdariffa*. However, *J. curcas* administration was more effective in restoring the urea and creatinine levels. Our results also revealed non-significant changes in the electrolytes (Na^+ , K^+ , Cl^- , and HCO_3^-) levels in the PbA treated rats compared to the normal control. Mouw et al. [29] had previously reported that renal tissue electrolytes were unaltered by lead. Our results were consistent with his findings.

Histological examination of renal sections in lead acetate (50 mg/kg) treated group showed the degeneration and necrosis in tubules and swelling in the glomerulus, as compared to control negative group. Glomerular and

tubular epithelial changes were mild in the group that received co-administration of PbA and aqueous extract of the 100 mg/kg *J. curcas* showed mild tubular epithelial changes while in case of animal treated with aqueous extracts of *H. sabdariffa* showed regeneration in tubular epithelial cells. We think that morphological changes in kidneys were because of lead acetate, but these changes tended to be considered mild in lead acetate plus plant extract treated groups. The restoration pattern was dose-dependent and showing different levels of protection in the rat tissues treated with *H. sabdariffa* aqueous extract. The present study reveals that the protective action of *J. curcas* and *H. sabdariffa* might be due to the interaction of the various vital phytoconstituents, such as flavonoids and polyphenolic compounds present in the plants.

5. Conclusions

The results suggest that the aqueous extract of *J. curcas* and *H. sabdariffa* might reduce lead acetate-induced nephrotoxicity by their antioxidant activities. Further experiments are however needed to determine their exact mechanism of protection.

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