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# Hepato-Renal Effect Of *Solanum Anomalum* Thonn. Ex. Schumach Fruit Extracts on Lead-Exposed Albino Rats

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## ABSTRACT

Lead is a non-biodegradable multi-organ toxicant implicated in various disorders including renal and hepatic diseases. In the quest for a cheaper and readily available antidote, this study has investigated the role of *Solanum anomalum* fruit extract in lead-induced kidney and liver toxicities in male albino rats. Twenty-four mature male albino rats were used, divided into four groups of six rats per group. Group 1 (control rats) received distilled water (10 ml/kg), group 2 was given lead acetate solution 60 mg/kg, group 3 rats were given lead acetate (60 mg/kg) followed by *Solanum anomalum*, SA (452 mg/kg) and group 4 rats were given lead acetate (60 mg/kg) followed by *Solanum anomalum*, SA (678 mg/kg) by oral gavage daily for 28 days. Animals in the lead acetate-treated group showed significant increases in ALT, AST, ALP, urea, bilirubin, total cholesterol, triglycerides, Low Density Lipoprotein, Very Low Density Lipoproteins, total white blood cell counts, Interleukin-6, and decreases in body weight, packed cell volume, hemoglobin concentration, red blood cell count, total proteins and albumins. Co-administration of *Solanum anomalum* fruit extract significantly reversed most of these biomarkers. Histopathology of kidney and liver also points to the protective effect of *Solanum anomalum* fruit against lead induced hepato-renal damage.

**Keywords:** lead, liver, kidney, *Solanum anomalum*, biomarkers, toxicant.

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## INTRODUCTION

Lead is a major cumulative toxicant that affects various organs, causing harm particularly in children.[1] Primarily, it affects the central nervous system, hepatic, renal and haematopoietic systems causing serious disorders.[2] There is currently no known safe blood lead concentration. Even blood lead concentration as low as 5 mg/dL, once considered safe, may result in decreased intelligence in children, behavioural difficulties and learning problems.[3] Older children and adults are vulnerable to the severe consequences of prolonged exposure to lead, including increased risk of cardiovascular death and kidney damage later on in life.[4] Lead exposure from the environment has been reported as a major risk factor for hypertension and a possible risk factor for mortality resulting from cardiovascular disease.[5,6] In humans, higher blood lead levels have also been associated with electrocardiographic abnormalities, peripheral arterial disease and left-ventricular hypertrophy.[4,7,8] Excessive dietary intake of lead has been linked with cancers of the kidney, stomach, small intestine, large intestine, ovary, lungs, myeloma, all lymphomas, and all leukemia.[9] Acute, high dose lead exposures may result in gastrointestinal disturbances, such as anorexia, nausea, vomiting and abdominal pain; neurological effects, such as malaise and drowsiness, as well as hepatic and renal damage.[10] Latest report from the Institute for Health Metrics and Evaluation (IHME) shows that approximately 815 million children are estimated to have blood lead levels above 5µg/dL globally and over 900,000 premature deaths per year in adults,[11] including 21.7 million years of healthy life lost (measured in Disability Adjusted Life Years or DALYs) as a result of the long-term effects of lead exposure on health.[12] Exposure to lead accounted for about 2.9 per cent of the global burden of chronic kidney disease, 62.5 per cent of the global burden of idiopathic developmental intellectual disability, 8.2 per cent of the global burden of hypertensive heart disease, 4.6 per cent of the global burden of ischemic heart disease and 4.7 per cent of the global burden of stroke.[13] About 92 per cent of all deaths attributable to lead exposures occur in low- and middle-income countries.[14]

Children from poor nations are particularly vulnerable to the harmful effects associated with lead poisoning such as those with family members

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working in industries such as artisanal ceramic workshops, metal mines, informal smelting and lead-acid battery recycling without protective equipment.[15] Such children do not have access to good and qualitative healthcare[16] and have poor nutritional status such as deficiencies in calcium and iron that can result in increased absorption of lead.[17,18,19] The mainstay of treatment of lead poisoning is chelation therapy. Chelating agents contain sulfhydryl groups which bind or chelate lead to form a complex that is excreted either renally or hepatically. The pharmaceutical Chelating agents, e.g. Succimer and Penicillamine are administered orally, whereas Dimercaprol and edetate (EDTA) calcium disodium ($CaNa_2$ EDTA) are administered parenterally. In addition to reducing blood lead level these classic chelating agents may also mobilize skeletal stores of lead thereby causing their redistribution and toxicity to other organs like the brain as well as causing other adverse effects.[20] In addition to the adverse effects, the prohibitive cost and scarcity of these agents pose serious management challenge in resource poor countries in the developing nations. Hence, the need to explore readily available and natural antidote in the management of lead poisoning.

The plant, *Solanum anomalum:* Thonn. Ex. Schumach is about 2 meters in height with prickles up to 5mm long on stem, branches and midrib of the leaves. The fruit is a ball-shaped berry of 5-9 mm in diameter, shiny red when mature and green when young. The seeds are also globose 2-3 mm in diameter and ball-shaped.[21] The mature red fruits of *Solanum anomalum* are harvested from the wild and made into soups and sauces, or can be eaten fresh. The exudate from the leaves and fruits is drunk or taken by enema 1-2 times daily as a treatment for leprosy in West Africa. In Nigeria, the fruit is used as a laxative and appetizer. Anecdotal evidence laid claim to the usefulness of the fruits in the treatment of splenomegaly in children.[22] The locals also claim that eating the raw fruits help to treat malaria. The leaf exudate has been used to treat gonorrhea, and crushed fruits extracts are used as anti-inflammatory and analgesic.[23] Very few scientific works have been done on the plant. This fruits contain saponins, cardiac glycosides, anthraquinones, terpenes, flavonoids, tannins and alkaloids.[22] The LD50 was reported as 2260 ± 131.78mg/kg, in addition to the anti-diabetic effect of the ethanolic extract and fractions of the fruits.[22] In another study, Offor et al.[24] reported that *Solanum anomalum* fruit extract may be a protective modulator of lead-induced testicular injury. The present study seeks to examine the protective effect of *Solanum anomalum* fruit extracts on lead-induced liver and kidney injuries in albino rats.

## MATERIALS AND METHODS

Chemicals: Lead acetate trihydrate (May & Baker, England) was dissolved in distilled water; Thiobarbituric acid, eosin, formalin and hematoxylin (Merck, Germany), Rat ELISA (Enzyme-linked immunosorbent assay) kits (RayBiotech, Inc. USA and Assaypro LLC, USA)

Animal husbandry: Twenty-four male albino Wistar rats, weighing 145 – 170 g (aged 11-15 weeks) obtained from the University of Uyo Animal house, were acclimatized for two weeks, maintained under controlled conditions of temperature (23 ± 2°C) and humidity (50 ± 5%) and a 12-h light–dark cycle, were used for the experiment (31). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. The bedding of the cages was changed weekly, and the cages were cleaned as well. They had free access to standard rat pellet diet and water ad libitum.[25] The procedures were performed according to the guidelines on the use of animals and approved by the Institutional Animal Ethical Committee of the University of Uyo, Nigeria (Ethical Approval No: UNIUYO/PHARM/2015/0153).

### Plant collection

*Solanum anomalum* with the fruits was obtained from a farmland in Obot Ndom Itumbonuso village, Ini Local Government Area of Akwa Ibom State, Nigeria. It was identified and authenticated by Professor M.E.Bassey, a plant taxonomist in the Department of Botany and Ecological Studies, University of Uyo. The plant specimen (voucher number UUH: No 75(a)) was deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.

### Preparation and Extraction

The fruits were separated from the stalk and air dried under room temperature for 3 weeks. The dried fruits were powdered using pestle and mortar. The extract was prepared by maceration (cold extraction) of 350.05g of the air-dried, powdered fruits of *S. anomalum* using 60% ethanol in distilled water (v/v) in an extracting jar. This set up was allowed to stand for 72 hours with occasional shaking. The extract was filtered, concentrated until constant weights were achieved and stored in a refrigerator at 2-8°C for use in subsequent experiments. This procedure was repeated 3 times for maximal extraction (yield 69.8%). The LD50 of *Solanum anomalum* was determined by Offor & Ubengama[22] as 2260 ± 131.78 mg/kg. The chosen

doses were the middle dose (20% of the LD50) which is 452mg/kg and the high dose (30% of the LD50) which is 678 mg/kg.

### Experimental design

The rats were divided into four groups of six rats per group as follows:

Group 1: Control rats: They were given distilled water (10ml/kg) by oral gavage daily for 28 days.
Group 2: Contained lead acetate solution 60mg/kg by oral gavage daily for 28 days [26]
Group 3: Lead acetate (60 mg/kg), plus *Solanum anomalum* fruit extracts (452 mg/kg) by oral gavage daily for 28 days.
Group 4: Lead acetate (60 mg/kg), plus *Solanum anomalum* fruit extracts (678 mg/kg) by oral gavage daily for 28 days. In all animals received *Solanum anomalum* fruit extracts 90 minutes after administration of lead.

At the end of the experiment and 24 hours after the last dose, animals were weighed and blood samples collected.

### Blood sample collection

Blood sample was collected using the Orbital technique.[27] Blood was collected from the retro-bulbar plexus of the medial canthus of the eye of the rat.[28] The blood (without anticoagulant) was kept at room temperature for 30 minutes to clot. Afterwards, the test tube containing the clotted blood sample was centrifuged at 3,000 revolutions per minutes for 10 minutes. The clear serum supernatant was then carefully aspirated with syringe and needle and stored in a clean sample bottle at -20˚C until use for biochemical assay.

### Necropsy

Blood was collected by retro orbital sinus puncture.[27] Each blood sample was divided into two portions. The first one was mixed well with the anticoagulant, dipotassium EDTA by shaking and used for hematological screening. The second portion (without anticoagulant) was kept at room temperature for 30 min to clot. Afterward, the clotted blood sample was centrifuged at 3,000 rpm for 10 min. The clear serum supernatant was then carefully aspirated and stored in a clean sample bottle for the determination of some biochemical parameters. Rats were sacrificed under ether anesthesia[29] and concussion stunning involving manually applied trauma on the head;[30] the kidney and liver were excised, weighed, rinsed in saline, and preserved in 10% formalin for histopathological study.

### Hematological Screening

Total White Blood Cell Counts (TWBC), Packed Cell Volume (PCV), Red blood cell (RBC) count, Lymphocytes% (L%) and Neutrophils% (N%) were determined using the Hemocytometer method.[31] Hemoglobin (Hb) concentration was determined by the Cyanmethemoglobin method.[32]

### Biochemical Analysis

Total serum bilirubin,[33] serum total proteins,[34] serum albumins,[35] serum Globulin (calculated by subtracting the quantity of albumins from that of total proteins), serum total cholesterol,[36] High Density Lipoprotein (HDLs),[37] Low Density Lipoprotein (LDL) and Very Low Density Lipoproteins (VLDL),[38,39] serum triglycerides,[40] serum creatinine,[41] and serum urea,[42,43] were determined. The following liver enzymes were also assayed: Alanine Aminotransferase (ALT) and Aspartate aminotransferase (AST)[44] and Alkaline phosphatase (ALP).[45]

### Determination of Serum Levels of Pro-Inflammatory Cytokines

Serum levels of pro-inflammatory cytokines (Tumor necrosis factor-alpha, TNF-a) and Interleukin-6 (IL-6) were determined using rat ELISA (Enzyme-linked immunosorbent assay) kits in accordance with manufacturer's recommended protocols (RayBiotech, Inc. USA and Assaypro LLC, USA).

### Statistical Analysis

Results were expressed as mean ± standard deviation, SD. Statistical analysis was carried out with one way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test. Values of $p < 0.05$ were considered to be significant.

## RESULTS

### Effect of *Solanum anomalum* fruit extract on Body Weight, Absolute and Relative Organ Weights (liver and kidney) of Lead Acetate-treated Rats

The effects on body weight, absolute and relative weights of liver and kidney are shown on Table 1. Lead acetate administration led to significant decrease in body weight of rats (from 190.77g ± 10.95 to 164.37g ± 17.28). Administration of *Solanum anomalum* (452 and 678 mg/kg) after lead acetate, resulted in a dose-dependent changes in body weight of 172.98g±18.01 and 194.53g±27.24 respectively. The absolute and relative weights of liver in the control group were 5.97g ± 0.37 and 3.36 ± 0.19 respectively. In the lead acetate treated group, the absolute and relative weights of liver were

4.96g ± 0.14and3.00 ± 0.25 respectively; in the 452 mg SAgroup-6.33g±0.01 and3.49±0.23 respectively while that in the 678mg SA group were6.22g±0.04 and 3.40±0.02 respectively. Similarly, the absolute and relative weights of kidney in the control, lead acetate-treated, 452mg *Solanum anomalum* and 678mg *Solanum anomalum* groups were (0.52g ± 0.05, 0.35 ± 0.01); (0.61g ± 0.07, 0.35 ± 0.00); (0.64g±0.21, 0.36±0.01); and (0.58g±0.01, 0.34±0.00) respectively.

### Effect of *Solanum anomalum* fruit extract on Hematological Parameters

The effect on haematological parameters is as shown in Table 2. Treatment of rats with lead acetate (group2) caused significant ($p<0.05$) decreased in Packed cell volume (PCV), Haemoglobin (Hb) concentration and Red blood cell (RBC) count when compared to control. These parameters were increased significantly ($p<0.05$) in groups 3 and 4 animals which were given452 mg/kg and 678 mg/kg of the extract respectively, after lead acetate compared to the group given lead acetate only. The effect caused by the lower dose of the extract (452 mg/kg) was however, significantly different relative to control. Rats in group 2 (given lead acetate only) also had significant increase ($p<0.05$) in total white blood count (WBC) when compared to rats in the control group, while the total WBC in the groups treated with the two doses of the extract were significantly ($p<0.05$) decreased. However, there was no effect on lymphocyte and neutrophil percentages.

### Effect of *Solanum anomalum* fruit extract on Biochemical Parameters

Table 3 shows the effect of *Solanum anomalum* on the serum levels of AST, ALP, and ALT in lead acetate-treated male albino rats. Rats in group 2 showed significant ($p<0.05$) increase in these liver enzymes when compared with the normal control group (group 1).These liver enzymes were decreased dose-dependently in the two extract-treated groups. The effect of *Solanum anomalum* on serum total proteins, Albumins and Globulins in lead acetate-treated male albino Wistar rats is shown on Table 4. There was significant ($p<0.05$) decrease in serum total proteins and albumins in group 2 rats compared to those in group 1. These two parameters were increased significantly ($p<0.05$) in the group given the higher dose (678 mg/kg) of the extract. Animal group treated with 452 mg/kg of extract showed no significant ($p>0.05$) increase in total proteins when compared to the group treated with lead acetate only but caused significant ($p<0.05$) increase in albumins, though this increase is significantly ($p<0.05$) different from that of control. Serum level of globulin was however, not significantly ($p>0.05$) affected in all the groups compared to control.

There were significant ($p<0.05$) increases in urea and bilirubin in group 2 animals compared to that of rats in the control group. These parameters were decreased significantly ($p<0.05$) in the group treated with 678 mg/kg of the extract. The group treated with 452 mg/kg of extract also significantly ($p<0.05$) decreased the serum levels of bilirubin and urea but the decrease in urea level was not significant ($p>0.05$) when compared to the lead acetate-only group. There was no significant effect ($p>0.05$) on creatinine level in all groups (Table 5).

The effect on Lipid profiles is as shown in Table 6. Rats given lead acetate-only (group 2) showed significant ($p<0.05$) increase in total cholesterol, triglycerides, LDL and VLDL compared to those in the control group. The higher dose of the extract (678 mg/kg) significantly ($p<0.05$) decreased the levels of LDL, VLDL, triglycerides and total cholesterol when compared to the group treated with lead acetate only. The effect of this higher dose of extract was however, significantly ($p<0.05$) different relative to control. Lower dose of extract (452 mg/kg) caused significant ($p<0.05$) decrease in

**Table 1** **Effect of SA extract on body weight, absolute and *relative organ weights of lead acetate-treated rats**

| Group weight | Treatment | Body weight (g) | Absolute weight liver (g) | Relative weight liver | Absolute weight kidney (g) | Relative weight kidney |
|---|---|---|---|---|---|---|
| 1 (Control) | Distilled water (10ml/kg) | 190.77±10.95 | 5.97±0.37 | 3.36±0.19 | 0.52± 0.05 | 0.35±0.01 |
| 2 | Lead Acetate (60mg/kg) | 164.37[a]±17.28 | 4.96 ±0.14 | 3.00±0.25 | 0.61 ± 0.070 | 0.35±0.00 |
| 3 | Lead acetate (60mg/kg) ± SA (452 mg/kg) | 172.98±18.01 | 6.33±0.01 | 3.49±0.23 | 0.64±0.21 | 0.36±0.01 |
| 4 | Lead acetate (60mg/kg) ± SA (678 mg/kg) | 194.53±27.24 | 6.22±0.04 | 3.40±0.02 | 0.58±0.01 | 0.34±0.00 |

Data were expressed as mean ± SD.
a: significantly different when compared to the control group ($p< 0.05$) (n = 6)

**Table 2** **Effect of SA extract on haematological parameters of lead acetate-treated rats**

| Group | Treatment | Packed cell volume, PCV (%) | Haemoglobin, Hb concentration (g/dl) | Red blood cell count, RBC ($10^6$/µL) | Total WBC count ($10^3$/µL) | Lymphocyte (%) | Neutrophil (%) |
|---|---|---|---|---|---|---|---|
| 1(control) | Distilled water (10ml/kg) | 40.67 ± 1.63 | 16.31 ± 0.72 | 7.89 ± 0.18 | 19.24 ± 4.85 | 71.00 ± 3.35 | 26.83 ± 3.55 |
| 2 | Lead Acetate (60mg/kg) | 32.83 ± 1.29[a] | 12.57 ± 0.66[a] | 4.41 ± 1.05[a] | 30.66 ± 3.40[a] | 77.00 ± 6.42 | 26.33 ± 1.51 |
| 3 | Lead acetate (60mg/kg) ± SA (452mg/kg) | 37.75 ± 1.84[a,b] | 14.83 ± 0.69[a,b] | 6.76 ± 0.53[a,b] | 22.53 ± 3.51[b] | 73.67 ± 4.08 | 24.83 ± 1.84 |
| 4 | Lead acetate (60mg/kg) ± SA (678mg/kg) | 39.67 ±0.93[b] | 15.23±0.42[a,b] | 7.82±0.30[b] | 20.35±4.87[b] | 72.00±3.80 | 26.00±2.97 |

Data were expressed as mean ± SD.
a: significantly different when compared to the control group ($p<0.05$)
b: significantly different when compared to the lead acetate- treated group ($p<0.05$) (n=6)

**Table 3** **Effect of SA extract on the serum levels of Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP) and Alanine Amino Transferase (ALT) of Lead acetate-treated rats**

| Group | Treatment | AST (U/L) (U/L) | ALP (U/L) | ALT (U/L) |
|---|---|---|---|---|
| 1 (control) | Distilled water (10ml/kg) | 55.77 ± 0.88 | 180.79 ± 0.17 | 17.67 ± 2.05 |
| 2 | Lead Acetate (60mg/kg) | 76.77 ± 9.20[a] | 211.71 ± 9.63[a] | 35.53 ± 2.82[a] |
| 3 | Lead acetate (60mg/kg) ± SA (452mg/kg) | 57.81 ± 11.43[b] | 205.06 ± 6.50[a] | 25.67 ± 3.77[a,b] |
| 4 | Lead acetate (60mg/kg) ± SA (678mg/kg) | 56.60±1.54[b] | 195.37 ± 9.94[b] | 23.59±3.16[b] |

Data were expressed as mean ± SD.
a: significantly different when compared to the control group ($p<0.05$)
b: significantly different when compared to the lead acetate- treated group ($p<0.05$) (n=6)

**Table 4** **Effects of SA extract on Total proteins, Albumins and Globulins of lead acetate-treated rats**

| Group | Treatment | Total proteins (g/dl) | Albumins (g/dl) | Globulins (g/dl) |
|---|---|---|---|---|
| 1 (control) | Distilled water (10ml/kg) | 8.55 ± 0.34 | 4.28 ± 0.38 | 4.27 ± 0.35 |
| 2 | Lead Acetate (60mg/kg) | 6.61 ± 0.16[a] | 2.90 ± 0.17[a] | 3.71 ± 0.25 |
| 3 | Lead acetate (60mg/kg) ± SA (452mg/kg) | 7.37 ± 0.78[a] | 3.52 ± 0.25[a,b] | 3.85 ± 0.77 |
| 4 | Lead acetate (60mg/kg) ± SA (678mg/kg) | 8.21±0.40[b] | 3.95±0.11[b] | 4.26±0.31 |

Data were expressed as mean ± SD.
a: significantly different when compared to the control group ($p<0.05$)
b: significantly different when compared to the lead acetate- treated group ($p<0.05$) (n=6)

the levels of only two of these parameters (VLDL and triglyceride) relative to the lead acetate-treated group, although their values were significantly ($p<0.05$) different compared to control. Generally, there were no significant ($p>0.05$) effects on HDL. The effects on pro-inflammatory cytokines are shown in table 7. Rats in group 2 showed significant ($p<0.05$) increase in the pro-inflammatory cytokine, Interleukin 6 (IL-6) when compared to control. Level of this cytokine was significantly ($p<0.05$) decreased in the two doses of the extract. However, there was no effect on the serum level of tumor necrosis factor alpha, TNF-α.

## Histopathology of the Kidney and liver

### *Liver*

The histological photomicrograph of the liver tissue stained with H&E techniques of Group 1 (control) that received distilled water 10 ml/kg by oral gavage daily for 28 days is shown on Figures 1A & 1B.

**Table 5** **Effect of SA extract on the serum levels of Urea, Creatinine and Bilirubin of lead acetate-treated rats**

| Group | Treatment | Urea (mg/dl) | Creatinine (mg/dl) | Bilirubin (mg/dl) |
|---|---|---|---|---|
| 1 (control) | Distilled water (10ml/kg) | 17.92 ± 2.83 | 0.59 ± 0.08 | 0.27 ± 0.11 |
| 2 | Lead Acetate (60mg/kg) | 29.00 ± 3.23[a] | 0.64 ± 0.05 | 0.97 ± 0.31[a] |
| 3 | Lead acetate (60mg/kg) ± SA (452mg/kg) | 24.95 ± 5.81[a] | 0.63 ± 0.10 | 0.45 ± 0.09[b] |
| 4 | Lead acetate (60mg/kg) ± SA (678mg/kg) | 17.76±4.48[b] | 0.55±0.07 | 0.33±0.14[b] |

Data were expressed as mean ± SD.
a: significantly different when compared to the control group ($p<0.05$)
b: significantly different when compared to the lead acetate- treated group ($p<0.05$) (n=6)

**Table 6** **Effects of SA extract on lipid profiles of lead acetate-treated rats**

| Group | Treatment | Total cholesterol (mg/dl) | HDL(mg/dl) | LDL(mg/dl) | VLDL (mg/dl) | Triglyceride (mg/dl) |
|---|---|---|---|---|---|---|
| 1(control) | Distilled water (10ml/kg) | 53.29 ± 5.57 | 22.16 ± 6.22 | 26.30 ± 9.21 | 4.94 ± 0.47 | 24.17 ± 2.84 |
| 2 | Lead Acetate (60mg/kg) | 78.51 ± 13.66[a] | 22.62 ± 5.00 | 40.07 ± 4.92[a] | 22.64 ± 12.64[a] | 113.20 ± 63.17[a] |
| 3 | Lead acetate (60mg/kg) ± SA (452mg/kg) | 74.32 ± 9.69[a] | 26.07 ± 3.27 | 33.81 ± 7.53[a] | 14.03 ± 6.26[ab] | 75.70 ± 26.00[ab] |
| 4 | Lead acetate (60mg/kg) ± SA (678 mg/kg) | 68.40±4.94[ab] | 26.64±6.72 | 28.46±8.60[b] | 12.69±5.89[ab] | 58.33±24.20[ab] |

Data were expressed as mean ± SD.
a: significantly different when compared to the control group ($p<0.05$)
b: significantly different when compared to the lead acetate- treated group ($p<0.05$) (n=6)

**Table 7** **Effect of SA extract on Pro-inflammatory cytokines of lead acetate-treated rats**

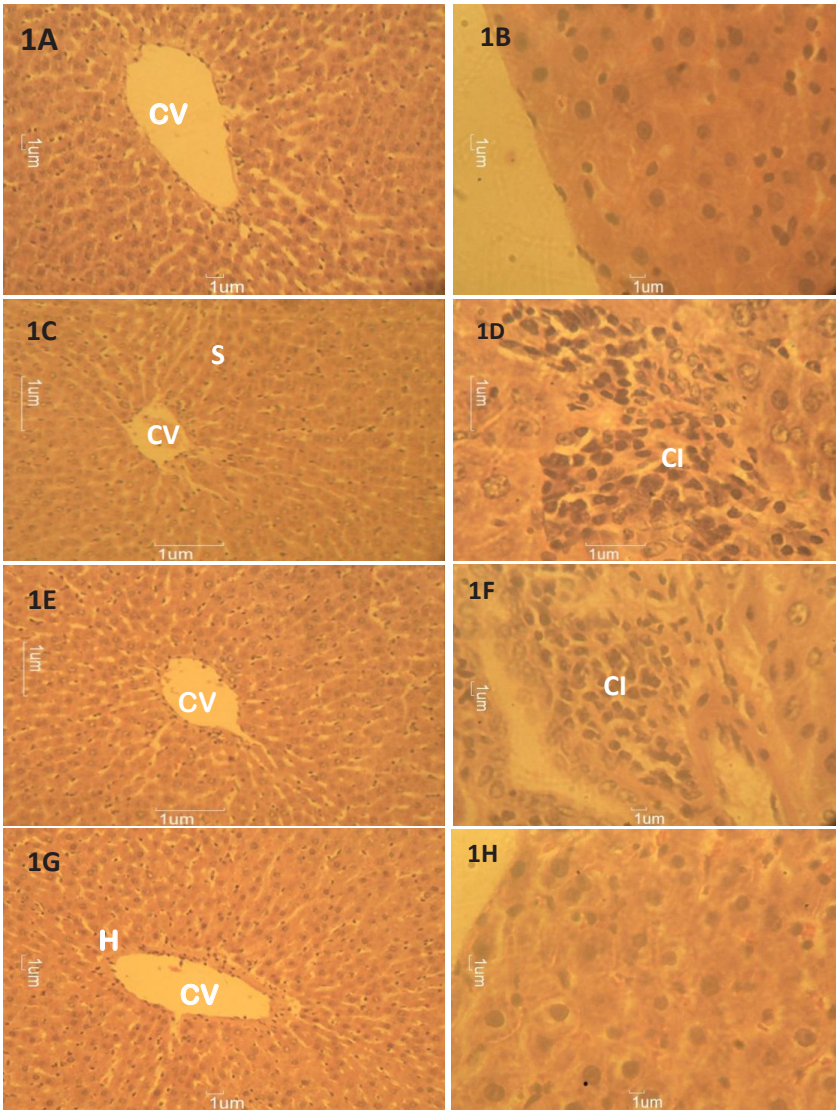
| Group Factor | Treatment | Interleukin 6 (IL-6) | Tumour Necrosis (TNF-α) |
|---|---|---|---|
| 1 (control) | Distilled water (10ml/kg) | 74.41 ± 5.45 | 0.01 ± 0.00 |
| 2 | Lead Acetate (60mg/kg) | 113.58 ± 13.46[a] | 0.00 ± 0.00 |
| 3 | Lead acetate (60mg/kg) ± SA (452mg/kg) | 81.68 ± 20.50[b] | 0.00 ± 0.00 |
| 4 | Lead acetate (60mg/kg) ± SA (678 mg/kg) | 76.65±7.18[b] | 0.01±0.00 |

Data were expressed as mean ± SD.
a: significantly different when compared to the control group ($p<0.05$)
b: significantly different when compared to the lead acetate- treated group ($p<0.05$) (n=6)

Liver tissue in this group shows normal central veins surrounded by intact hepatocytes with no vacuolar degeneration but only mild cellular infiltration. Figures 1C & 1D show the histological photomicrograph of the liver tissue stained with H&E techniques of Group 2 – Lead acetate 60 mg/kg daily for 28 days. Liver samples from this group (lead acetate-treated group) showed massive cellular (neutrophils and lymphocytes) infiltrations around the peri-portal areas, some hepatocyte necrosis and, vacuolar degeneration of peripheral hepatocytes. Figures 1E &1F show the histological photomicrograph of the liver tissue stained with H&E techniques of Group 3 – Lead acetate (60 mg/kg) followed by SA 452mg/kg) daily for 28 days. There was massive cellular infiltration of the peri-portal areas with lymphocytes and only a few vacuolated hepatocytes on the periphery. The histological photomicrograph of the liver tissue stained with H&E techniques of Group 4, lead acetate (60 mg/kg) followed by SA 678mg/kg) daily for 28 days is shown on Figures 1G & 1H. Liver tissues in this group were comparable to that in group 1, showing normal central veins surrounded by intact hepatocytes with no vacuolar degeneration and only mild cellular infiltration.

### *Kidney*

The histological photomicrograph of the kidney stained with H&E techniques of Group 1 (control) that received distilled water 10 ml/kg by oral gavage daily for 28 days is shown in Figure 2. A with normal




dilated tubules and glomeruli at the periphery of the cortex. Figures 2B & 2C show the histological photomicrograph of the kidney tissue stained with H&E techniques of Group 2 – Lead acetate 60 mg/kg daily for 28 days. The kidney tissue in this group shows degeneration and necrosis of the renal parenchymal cells with massive inflammatory cells infiltration. The kidney of rats in Group 2 – Lead acetate 60 mg/kg daily for 28 days showed areas of degeneration and necrosis of the renal cortical parenchymal cells with massive lymphocytic cellular infiltration, with vacuolar degeneration of the peripheral interstitial cells of the cortex. Tissue sections from the kidneys of rats in Group 3 – Lead acetate (60 mg/kg) followed by SA 452 mg/kg daily for 28 days showed areas of lymphocytic cellular infiltration, haemorrhage, necrosis and mild vacuolar degeneration of the peripheral interstitial cells of the cortex, but the medullary tubules were normal (Figure 2D). The histological photomicrograph of the kidney tissue stained with H&E techniques of Group 4, lead acetate (60 mg/kg) followed by SA 678mg/kg) daily for 28 days is shown on Figures 2E & 2F. Kidney tissues show normal dilated tubules in the periphery of the cortex and normal glomeruli with light lymphocytic cellular infiltration.

## DISCUSSION

The ubiquity and non-biodegradability of lead coupled with its multi organ toxicity is a great public health concern. This toxicity of lead includes its effect on the liver and kidney. Toxic effects on kidneys are manifested as structural damage and

**Figure 1** (A) Histological photomicrograph of the liver tissue stained with H&E techniques of Group 1 (control) that received distilled water 10 ml/kg by oral gavage daily for 28 days – Central veins with normal, intact hepatocytes surrounding it (1A Mag × 160). (B) Histological photomicrograph of the liver tissue stained with H&E techniques of Group 1 (control) that received distilled water 10 ml/kg by oral gavage daily for 28 days – Intact hepatocytes on the periphery with no vacuolar degenerations (1B Mag × 640). (C) Histological photomicrograph of the liver tissue stained with H&E techniques of Group 2 – Lead acetate 60 mg/kg daily for 28 days – Massive cellular infiltration around the portal areas with some hepatocyte necrosis (1C Mag × 160). (D) Histological photomicrograph of the liver tissue stained with H&E techniques of Group 2 – Lead acetate 60 mg/kg daily for 28 days – Vacuolar degeneration of peripheral hepatocytes, massive cellular infiltration and hepatocyte necrosis (1D Mag × 640). (E) Histological photomicrograph of the liver tissue stained with H&E techniques of Group 3 – Lead acetate (60 mg/kg) followed by SA 452 mg/kgdaily for 28 days- Massive cellular infiltration of the peri-portal areas with lymphocytes (1E Mag × 160). (F) Histological photomicrograph of the liver stained with H&E techniques of Group 3 – Lead acetate (60 mg/kg) followed by SA 452 mg/kg daily for 28 days – Massive cellular infiltration of the peri-portal areas with lymphocytes and only a few vacuolated hepatocytes on the periphery (1F Mag × 640). (G) Histological photomicrograph of the liver stained with H&E techniques of Group 4 – Lead acetate (60 mg/kg) followed by SA 678 mg/kg daily for 28 days – normal central veins surrounded by intact hepatocytes(1G Mag × 160). (H) Histological photomicrograph of the liver stained with H&E techniques of Group 4 – Lead acetate (60 mg/kg) followed by SA 678 mg/kg daily for 28 days – mild cellular infiltration with no vacuolar degeneration(1H Mag × 640)

Key: CV-central vein, CI-cellular infiltration, s- sinusoid, H-hepatocytes and V- vacuolization.

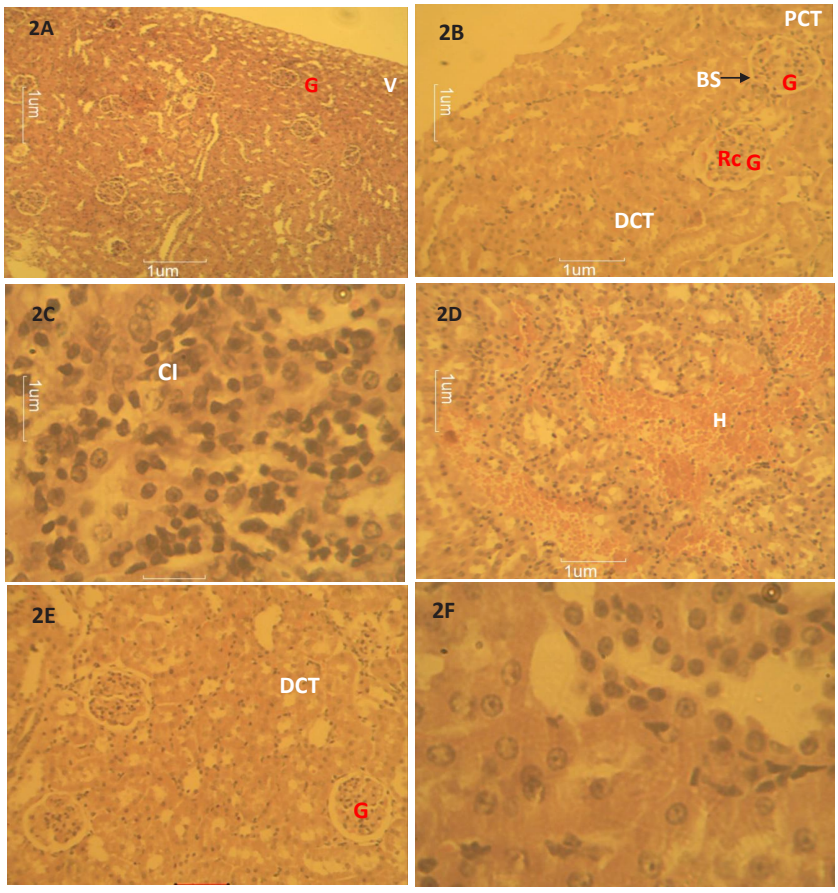



**Figure 2** (A) Histological photomicrograph of the kidney stained with H&E techniques of Group 1 – control that received distilled water 10 ml/kg via oral gavage daily for 28 days – Normal dilated tubules and glomeruli at the periphery of the cortex (2A Mag × 160). (B) Histological photomicrograph of the kidney tissue stained with H&E techniques of Group 2 – Lead acetate 60 mg/kg daily for 28 days – Degeneration and necrosis of the renal parenchymal cells (2B Mag × 160). (C) Histological photomicrograph of the kidney tissue stained with H&E techniques of Group 2 – Lead acetate 60 mg/kg daily for 28 days – Massive lymphocytic cellular infiltration, with degeneration and necrosis (2C Mag × 640). (D) Histological photomicrograph of the kidney stained with H&E techniques of Group 3 – Lead acetate (60 mg/kg) followed by SA 452 mg/kg daily for 28 days –Showed areas of lymphocytic cellular infiltration, haemorrhage, necrosis and mild vacuolar degeneration of the peripheral interstitial cells of the cortex, normal medullary tubules (2D Mag × 160). (E) Histological photomicrograph of the kidney stained with H&E techniques of Group 4 – Lead acetate (60 mg/kg) followed by SA 678 mg/kg daily for 28 days – normal dilated tubules in the periphery of the cortex and normal glomeruli with light lymphocytic cellular infiltration (2E Mag × 160). (F) Histological photomicrograph of the kidney stained with H&E techniques of Group 4 – Lead acetate (60 mg/kg) followed by SA 678 mg/kg daily for 28 days – normal dilated tubules and normal glomeruli with mild lymphocytic cellular infiltration (2F Mag × 640).

Key: G-glomerulus, BS-Bowman's space, Rc- renal corpuscle, PCT-proximal convoluted tubule, DCT-Distal convoluted tubule, Ct-convoluted tubule, H-Haemorrhage

changes in the excretory function.[46,47,48] Lead that is absorbed is conjugated in the liver and passed to the kidney, where a small amount is excreted in urine and the rest accumulates in various body organs and affects many biological activities at the molecular, cellular and intercellular levels, resulting in morphological changes that persist even after the levels of lead have fallen.[49] *Solanum anomalum* fruit extract is investigated in this study as a possible alternative to the classical antidotes owing to their numerous limitations which include many adverse effects (painful, hepatotoxicity, gastrointestinal symptoms), difficulties in their administration, not readily available and being expensive, among others.

In this study, lead acetate administration resulted in a significant decrease in body weight, while the administration of *Solanum anomalum* (452 and 678 mg/kg) after lead acetate resulted in a dose-dependent changes in body weight but no significant changes in the absolute and relative organ weights of the liver and kidney. The alteration in overall body weight or organ-body weight ratio is an indication of impairment in the normal functioning of the organs. Body weights are widely accepted for the evaluation of test-articles associated toxicities.[50] Treatment of rats with lead acetate in this study also caused significant reduction in packed cell volume (PCV), Haemoglobin (Hb) concentration and Red blood cell (RBC) count. Results of this study are in agreement with the findings of Nabil *et al.*,[51] Hanan and Riham. On the other hand, total WBC was significantly increased. Administration of the two doses of the extract (452 mg/kg and 678 mg/kg) respectively, caused significant increases in PCV, Hb and RBC as well as significant decrease in WBC. Lead directly affects the haematopoietic system by restraining the synthesis of haemoglobin through inhibition of various key enzymes involved in the heme synthesis pathway, particularly the enzyme Aminolevulinic Acid Dehydratase (ALAD). It also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes. The aftermaths of these two processes result in anaemia.[53,54]

Treatment of rats with lead acetate caused significant increase in the activity of serum AST, ALT, ALP, bilirubin and urea, while the levels of albumin and total proteins were decreased. Similar results were reported by Azoz and Raafat,[55] Ibrahim et al.[56] and Azab.[57] These parameters were however, reversed by treatment with the two doses of *Solanum anomalum* extract used in this study. Increasing levels of AST and ALT in the animals treated with lead acetate signify damage to the structural integrity of the liver. It is mainly the result of leakage of

these enzymes from the liver cytosol into the blood stream.[58] Releasing of AST and ALT from the cell cytosol can occur as secondary changes to cellular necrosis.[59] The high AST and ALT activities are accompanied by high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue.[56] Elevated level of ALP suggests biliary damage or an obstruction of the biliary tree, which disrupts the flow of blood to the liver.[60] The decrease in serum levels of these enzymes may be due to the prevention of their leakage from the liver cytosol by *Solanum anomalum*, probably due to reduction in blood lead level. The increase of bilirubin values in animals treated with lead acetate in this study may be the result of excessive heme destruction and blockage of biliary tract resulting in inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes.[61]

In this study, administration of lead acetate caused significant increase in serum urea level, but only a slight increase in serum creatinine level. Serum urea was significantly decreased with the respective administration the two doses of *Solanum anomalum*, but only a slight decrease in the value of creatinine. Urea and creatinine levels are generally used as suitable indicators of renal function. Creatinine is a break down product of creatine phosphate in muscle and for the most part, creatinine is cleared from the blood entirely by the kidney. Decreased kidney creatinine clearance results in a rise in blood creatinine. On the other hand, urea is the major nitrogenous end product of protein and amino acid catabolism which is produced by the liver.[62] Creatinine and urea elevation are biomarkers of abnormal renal function. However, serum creatinine is a later indicator of renal impairment as renal function is decreased by 50% before a rise in serum creatinine is observed.[63]

Lead acetate caused a significant decrease in the values of serum total proteins and Albumins. Supplementation with 678 mg/kg of *Solanum anomalum* in this study was able to significantly increase these parameters. The lower dose of the extract (452 mg/kg) also caused significant increase in albumin level. Decrease in serum total protein may be the result of hepatic and renal damage induced by lead,[64] or binding of lead to plasma proteins, leading to alteration in a high number of enzymes and can also disturb protein synthesis in hepatocytes.[65] Low level of serum total protein has also been attributed to a reduction in hepatic DNA and RNA induced by lead intoxication or as a result of decreased utilization of free amino acids for protein synthesis.[66] Albumin is the body's major serum-binding protein synthesized only in hepatic cells. Hypoalbuminaemia is due to several conditions such as nephrotic syndrome, hepatic cirrhosis, heart failure, and malnutrition. However, most cases of hypoalbuminaemia are due to acute and chronic inflammatory responses.[67] In this study, administration of lead acetate was found to cause elevation of Total cholesterol, triglycerides, LDL and VLDL. This result agrees with that reported by Azoz and Raafat.[55] The high lipid levels could stem from either increased synthesis or decreased removal of lipoproteins. Decreased removal may be due to the alteration of cell – surface receptors for lipoprotein.[68] or as a result of the inhibition of hepatic lipoprotein lipase activity.[69] The higher dose of the extract (678 mg/kg) caused significant reduction in all four lipid parameters mentioned above, while the extract's lower dose (452 mg/kg) only decreased the levels of triglyceride and VLDL. Although the two doses of the extract did not show any significant effect on HDL in this study, their effects in significantly lowering the high values of LDL, triglycerides and total cholesterol may reduce the risk of development of heart disease. Administration of lead acetate caused significant increase in the pro-inflammatory cytokine, Interleukine-6, IL-6. The serum level of another pro-inflammatory cytokine, Tumour necrosis factor-alpha (TNF-alpha) was undetectable in this study. However, administration of fruit extracts of *Solanum anomalum* significantly reduced the level of IL-6. Hence, the extract of *Solanum anomalum* could protect from lead acetate-induced toxicity by attenuating the increased serum IL-6. Measurements of cytokines and other inflammatory biomarkers can provide predictive clinical information and insights into disease mechanisms.[70] In our previous study, one of the mechanisms of protection by *Solanum anomalum* fruit extract involved elevation of antioxidant enzymes level to mitigate lead-induced reactive oxygen species, ROS that can cause oxidative organ damage.[24] This was attributable to its content of bioactive ingredients, including flavonoids. The liver and kidney of rats treated with lead acetate followed by supplementation with *Solanum anomalum* fruit extract demonstrated marked improvement in their histoarchitecture and with less pathological changes in comparison to the lead acetate group.

## CONCLUSION

In conclusion, *Solanum anomalum* fruits tend to be protective against liver and kidney injuries induced by chronic exposure to lead. Modulation of Interleukin-6, a pro-inflammatory cytokine and mitigation of lead-induced alteration in some biochemical and hematological parameters

following administration of the extract, may form part of the mechanisms of hepato-renal protection. Further studies may be necessary to elucidate the precise mechanism(s) of action.

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