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Full Length Article

# Polyphenol-rich Fraction of *Terminalia catappa* Prevents Chronic Lead Acetate Induced Oxidative Stress and Cardiorenal Toxicities in Rats

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ABSTRACT

*Background:* Lead (Pb), a naturally occurring environmental contaminant, has been implicated in several pathological conditions of the cardiovascular and renal systems.
*Objective:* The study was designed to evaluate the modulatory roles of the polyphenol-rich fraction of *Terminalia catappa* on chronic lead acetate-induced cardiovascular and renal toxicities in rats.
*Methods:* Thirty-six rats were randomly selected and divided into six groups of six rats each. Pb toxicity was induced by the administration of 100 mg/L Pb in drinking water for 12 weeks in groups B-F. Groups A and B were left untreated; groups C and D were treated with polyphenol-rich fraction of *Terminalia catappa* [PRFTC (100 and 200 mg/kg b.w.)]; vitamin E (50 mg/kg b.w.) and lisinopril (10 mg/kg b.w.) were administered to groups E and F, respectively.
*Results:* Exposure of rats to Pb induced significantly elevated ($P < 0.05$) primary haemodynamic parameters, severe disseminated congestion of blood vessels and haemorrhages in the cardiac and renal tissues, significantly elevated ($P < 0.05$) markers of oxidative stress markers of inflammation and myocardial infarction, but significantly decreased serum nitric oxide and the systemic antioxidants. Furthermore, rats exposed to Pb showed heightened immune-positive reactions to Caspase-3, a marker of apoptosis, in both renal and cardiac tissues. All manifestations of Pb-associated toxicities in the cardiovascular and renal systems were alleviated by the PRFTC treatment in rats.
*Conclusion:* The polyphenol-rich fraction of *T. catappa* proved effective in the reduction of oxidative stress-mediated derangements of the physiological homeostasis and decreased apoptosis in the cardiovascular and renal systems of rats chronically exposed to lead acetate toxicities and may therefore have therapeutic potential as a supplement that can be applied in chronic lead poisoning.

## 1. Introduction

Lead (Pb), one of the heavy metals with no nutritional value, has a lot of negative effects on biological systems and organs (Jamesdaniel et al., 2018). Despite continuous regulations by various agencies globally, sub-toxic levels of Pb exposures, which may become problematic over a long period, continue to occur due to unrelenting use of low Pb levels in household products and through environmental exposure via inhalation of air or dust, as well as food and water contamination (Kim et al., 2020). Most common Pb pollution sources are water pipes made of Pb, soldering wire, Pb-based paintings, ceramic screens, food packages, pastry powder, Pb painting sheets, and agriculture products enriched by fertilizers, fungicides and herbicides (Karimfar et al., 2016). In a recent report, Pb exposure accounted for 9.3% of the global burden of idiopathic intellectual disability, 4% of the global burden of ischemic heart disease and 6.6% of the global burden of stroke (Grover and Jhandar, 2017). Long term exposure to Pb even at low concentrations has been reported as a potential factor that induces hepatic, behavioral, reproductive, cardiovascular and renal dysfunctions (Chen et al., 2019).

Although Pb is normally distributed in various mammalian organs and tissues including the bones, blood, brain, liver and kidney, with varying levels of toxic manifestations, specific life-threatening toxicities in the heart and kidney are known to occur and are reportedly associated with chronic Pb exposure (Ustinova et al., 2015). The kidney is arguably one of the most susceptible organs to toxic manifestations of Pb because of its ability to reabsorb and accumulate the heavy metal during the excretory process (Flora and Gupta, 2012). For instance, acute and chronic exposure to Pb has been severally reported to cause severe pathologic manifestations in the kidney including renal proximal tubular dysfunction, chronic interstitial nephritis, irreversible progressive kidney disease and end stage renal failure (Zhou et al., 2016). Pb

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readily passes into the glomerular filtrate, reabsorbed in the proximal tubular cells, where it causes mitochondrial damage, uncoupling of the respiratory chain and cell death (Wang et al., 2009). Also, significantly increased blood pressure, endothelial nitric oxide synthase protein expression, and vascular reactivity to angiotensin II in rats exposed to Pb has been reported (Robles et al., 2007). In the same vein, chronic Pb exposure has been reported to cause persistent hypertension in genetically normal animals via the induction of oxidative stress, alteration of the renin-angiotensin-aldosterone system and impairment of the vascular smooth muscle compliance (Bravo et al., 2007). The sulfhydryl group of enzymatic antioxidant molecules including the superoxide dismutase, catalase, glutathione peroxidase and catalase readily bind Pb which significantly reduce their potency and disrupt the physiological equilibrium between the prooxidants and the antioxidants systems in favour of the prooxidants; as a result, Pb promotes oxidative stress in tissues (Wang et al., 2013). The mechanism of toxicity of Pb on sulfhydryl-dependent enzymes involves the formation of multidentate complexes with thiol (–SH) and other functional groups in the enzymes leading to a disruption of the normal enzymatic activity (Sharma et al., 2008).

Considering the prominent role of oxidative stress in Pb-induced toxicities, therapeutic strategies that enhance the antioxidant defense system may help prevent and/or ameliorate Pb-induced organ toxicities. Moreover, biological compounds, many of which are polyphenolic compounds of plant origin, with antioxidant properties are known to contribute to protection of cells and tissues against deleterious effects of reactive oxygen species and other free radicals (He et al., 2017; Abdel-Daim et al., 2020). Also, a number of plants such as *Moringa oleifera* and *Cyperus esculentus* have been reported to potently ameliorate Pb induced organ toxicities (Baty et al., 2020; Udefa et al., 2020). The leaf of *Terminalia catappa* Linn, family *Combretaceae*, is reported to possess strong antioxidant properties and is used in traditional medicine for the treatment of various ailments including hypertension (Akharaiyi et al., 2011). Different preparations of *T. catappa* leaf reportedly reduce serum cholesterol, serum triglycerides, serum low-density lipoprotein (LDL), serum creatinine and serum urea; thus suggesting a positive modulatory role for this plant in cardiovascular and renal system functioning. In addition, the antiinflammatory, antiangiogenic, antioxidant and radical scavenging activities of the plant have been reported (Fan et al., 2004). Therefore, this study was designed to exploit the potent antioxidant properties of the polyphenol-rich fraction of *T. catappa* (PRFTC) as a preventive therapeutic strategy in an *in vivo* model of cardiac and renal oxidative stress. Results from this study may provide a scientific basis for the use of this botanical as an effective drug for clinical prevention of Pb toxicity.

## 2. Materials and Methods

### 2.1. Experimental design and animal treatment

Thirty-six adult male rats of the Wistar strain (180.0 ± 5.0 g) were obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria and were used for this study. The animals were kept in wire mesh cages under controlled light cycle (12 h light/12 h dark) and fed with commercial rat chow *ad libitum.* They were liberally supplied with water. Acclimation of experimental rats to laboratory conditions for 14 consecutive days was done before the commencement of the experiment and the rats were subjected to humane care. Following the acclimation period, thirty six rats were randomly selected and divided into six groups of six rats each. Pb toxicity was induced by the administration of 100 mg/L Pb in drinking water for 12 weeks in groups B-F. Groups A and B were left untreated; groups C and D were treated with polyphenol-rich fraction of *Terminalia catappa* [PRFTC (100 and 200 mg/kg b.w.)] (Punniyakotti et al., 2019); vitamin E (50 mg/kg b.w.) and lisinopril (10 mg/kg b.w.) were administered to groups E and F, respectively. Vitamin E and lisinopril were used in this study based on previous report on their ability to mitigate oxidative stress-mediated pathogenic processes and positively modulate endothelial dysfunctions in rats (Ajibade et al., 2021).

### 2.2. Preparation of plant extract

The fresh leaves of *T. catappa* were collected from the Botanical Garden, University of Ibadan, Nigeria, and authenticated at the Herbarium of the Department of Botany, University of Ibadan. Five hundred grams of the air-dried, pulverised *T. catappa* leaf was extracted with distilled methanol by means of cold extraction as previously described (Njar et al., 1993). Following concentration in a rotary evaporator at 45°C , the solvent remaining in the extract was removed in a temperature-controlled oven to give a residue weighing 68 g (a yield of 13.6%). Thereafter, the residue was partitioned with ethylacetate to give an ethylacetate fraction that was subsequently evaporated under reduced pressure and dried using a temperature-controlled oven to obtain the polyphenol-rich fraction of *T. catappa*.

### 2.3. Chemicals

All chemicals used for this study were of analytical grade. They include glutathione, hydrogen peroxide, sodium hydroxide, epinephrine, xylenol orange, 1,2-dichloro-4-nitrobenzene (CDNB), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), thiobarbituric acid (TBA) (Sigma, St. Louis, MO, USA). Normal goat serum, Biotinylated antibody, and Horse Rdish Peroxidase (HRP) System was purchased from *KPL, Inc.*, Gaithersburg, Maryland, USA. Caspase-3 antibody was purchased from *Bioss Inc.* Woburn, Massachusetts, USA. Diaminobenzidine (DAB) tablets were purchased from Amresco LLC. Ohio, USA.

### 2.4. Blood pressure measurement

The blood pressure readings were taken in non-anaesthesised rats by tail plethysmography, using an electrosphygnomanometer (CODA, Kent Scientific, USA). The rats were placed in a warm chamber, 35°C , and trained for 10 min before the commencement of blood pressure measurement. The average of 20 consistent readings, taken in the most quiescent state of the rats, was recorded.

### 2.5. Isolation of post-mitochondrial fraction and biochemical analysis

Three millimitres of blood was collected from the retro-orbital venous plexus of rats into heparinised sample bottles prior to the sacrifice of each rat. The blood collected into non-heparinised tubes was allowed to clot, centrifuged and the serum decanted. Following humane sacrifice, the kidney and heart of the rats were harvested, rinsed with normal saline and homogenized on ice in aqueous potassium buffer (pH 7.4, 0.1 M). The homogenate was centrifuged at 12,000 rpm, in a cold centrifuge, for 15 min to obtain the supernatant fraction. Subsequently, the serum and post mitochondrial fractions were stored at 4°C . The activity of xanthine oxidase was determined according to the method described by Akaike et al. (Akaike et al., 1990). Nitric oxide was quantified as described by Olaleye et al. (Olaleye et al., 2007), while the thiol contents were estimated by the method of Ellman (Ellman, 1959). The reduced glutathione (GSH) concentration was estimated as previously described by Jollow et al. (Jollow et al., 1974). Glutathione peroxidase (GPx) and Glutathione-S-transferase (GST) activities were measured as described by Rotruck et al. (Rotruck et al., 1973) and Habig et al. (Habig et al., 1974), respectively. Also, AOPP levels were determined as previously described by Kayali et al. (Kayali et al., 2006).

Superoxide dismutase (SOD) was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 as previously described by Misra and Fridovich (Misra and Fridovich, 1972). The malondialdehyde (MDA) level was calculated as described by Varshney and Kale (Varshney and Kale, 1990). Hydrogen peroxide ($H_2O_2$) generation was estimated as described by

**Table 1**
Effects of *Terminalia catappa*, Vitamin E and lisinopril on cardiac antioxidants.

| Parameters | Control | Lead | Lead + Extract 100 mg/kg | Lead + Extract 200 mg/kg | Lead + Vitamin E | Lead + Lisinopril |
|---|---|---|---|---|---|---|
| GPx | 145.4 ± 11.9 | 127.7 ± 4.7[a] | 156.9 ± 0.9 [b] | 156.6 ± 6.0 [b] | 157.0 ± 5.75 [b] | 164.5 ± 14.3 [b] |
| GST | 321.2 ± 4.9 | 248.1 ± 4.0 [a] | 322.6 ± 23.8 [b] | 324.9 ± 6.6 [b] | 324.9 ± 12.8 [b] | 317.0 ± 7.8 [b] |
| GSH | 66.1 ± 1.7 | 48.4 ± 2.7 [a] | 71.2 ± 0.9 | 72.6 ± 1.7 [b] | 73.2 ± 2.33 | 74.3 ± 2.2 [b] |
| Total Thiol | 37.3 ± 0.6 | 30.6 ± 0.6 [a] | 31.5 ± 2.0 | 31.7 ± 1.3 | 32.6 ± 0.5 [b] | 33.5 ± 1.1 [b] |

[a] Superscript (a) indicates significant decrease compared with Control at $P < 0.05$; Superscript (b) indicates significant increase compared with toxicant (lead exposed) groups at $P < 0.05$. GPx (Glutathione peroxidase, units/mg protein); GST (Glutathione-S-transferase; mmole/min/mg protein); GSH (Reduced Glutathione; micromole/g tissue); SOD (Superoxide dismutase; units/mg protein); TOTAL THIOL (nmole/mg protein).

Wolff (Wolff, 1994). Protein concentration was determined by Biuret method as described by Gornal et al. (Gornal et al., 1949). The serum myeloperoxidase (MPO) activity was determined according to the method of Xia and Zweier (Xia and Zweier, 1997).

### 2.6. Histopathology

Cut portions of heart and kidney tissues were fixed in 10% formalin, processed, and embedded in paraffin wax. Five to six micrometer thick sections were made and stained with Haematoxylin and Eosin for histopathological examination (Drury, 1976).

### 2.7. Immunohistochemistry of Caspase-3

The paraffin embedded heart and kidney tissues were processed for immunohistochemistry based on the methods described by Oyagbemi et al. (Oyagbemi et al., 2017). Briefly, paraffin sections were melted at 60°C in the oven. The tissues were dewaxed in xylene, and subsequently passed through ethanol of decreasing concentration (100 to 80%). Peroxidase quenching in 3% hydrogen peroxide/methanol was carried out with subsequent antigen retrieval performed by microwave heating in 0.01 M citrate buffer (pH 6.0). Thereafter, the sections were blocked in normal goat serum and probed with Caspase-3 antibody, 1:200 for 16 h. Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0 µg/mL) secondary antibody and streptavidin peroxidase (Horse Radish Peroxidase-streptavidin) according to the manufacturer's instruction (HistoMark®, KPL, Gaithersburg MD, USA). The reaction product was enhanced with diaminobenzidine (DAB, Amresco®, USA) for 6–10 min, counterstained with high definition hematoxylin (Enzo®, NY-USA), and dehydrated with ethanol. The slides were covered with cover slips and sealed with resinous solution. The immunoreactive positive expression of Caspase-3 intensive regions were viewed starting from low magnification on each slice, then with 400× magnifications using a photo microscope (Olympus) and a digital camera (Toupcam®, Touptek Photonics, Zhejiang, China).

### 2.8. Statistical analysis

All values were expressed as Mean ± Standard Deviation (SD). The test of significance between two groups was estimated by the Student's *t*-test. One-way Analysis of Variance (ANOVA) with Tukey's post-hoc test using Graph pad prism 5.0 was also performed, and *P* values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Effects of lead exposure on cardiac and renal antioxidant defence system

Exposure to Pb caused a significant reduction in the activities of cardiac GPx, GST and SOD (Table 1). Rats treated with PRFTC, vitamin E and Lisinopril showed significant increase ($P < 0.05$) in the activities of GPx, GST and SOD. The cardiac GSH and total thiol contents declined significantly ($P < 0.05$) in Pb exposed rats compared to rats treated with PRFTC extract and vitamin E (Table 1). Similarly, exposure to lead acetate caused a significant reduction in the activities of renal GPx, GST and SOD when compared to rats treated with PRFTC and vitamin E (Table 1). The observed reduction in the GSH and total thiol contents of renal tissues was not statistically significant in all treatment groups (Table 2).

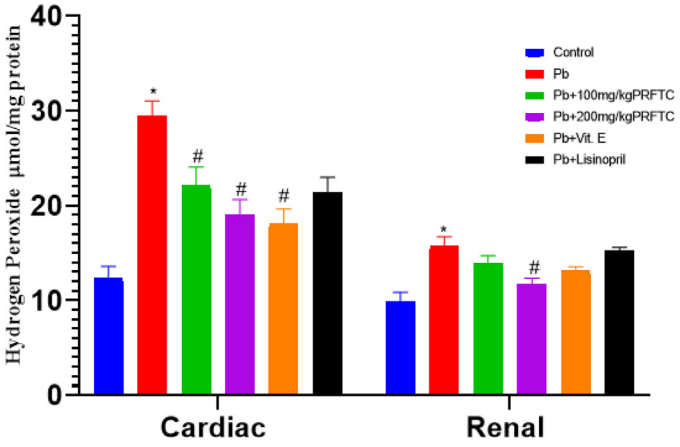


**Fig. 1.** Hydrogen peroxide in cardiac and renal tissues. Superscript (*) indicates significant increase compared with Control; superscript (#) indicates significant decrease compared with Pb.

### 3.2. Effects of lead acetate exposure on blood pressure parameters

Significantly higher ($P < 0.05$) systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) were recorded in PRFTC exposed rats following a twelve-week exposure period, but treatment of rats with PRFTC and vitamin E significantly reduced the SBP, DBP and MAP of hypertensive rats (Table 3). The changes observed on cardiac and renal absolute and relative organ weights were not statistically significant (Table 4).

### 3.3. Effects of PRFTC on cardiac and renal markers of oxidative stress

Following exposure to PRFTC, there was a significant increase ($P < 0.05$) in the contents of hydrogen peroxide ($H_2O_2$), malondialdehyde (MDA) and advanced oxidative protein products (AOPPs) in the cardiac and renal tissues of rats, compared to the rats treated with PRFTC, and vitamin E (Figs. 1, 2 and 3 respectively).

### 3.4. Effects of PRFTC on serum myeloperoxidase (MPO), xanthine oxidase (XO) and nitric oxide (NO)

Rats administered PRFTC alone had a significant increase ($P < 0.05$) in the serum MPO activity compared to other treatment groups (Fig. 4).

**Table 2**
Effects of *Terminalia catappa*, Vitamin E and lisinopril on renal antioxidants

| Parameters | Control | Lead | Lead + Extract 100 mg/kg | Lead + Extract 200 mg/kg | Lead + Vitamin E | Lead + Lisinopril |
|---|---|---|---|---|---|---|
| GPx | 62.5 ± 1.3 | 61.0 ± 3.5 | 71.4 ± 4.74[b] | 71.9 ± 2.2[b] | 73.9 ± 3.2[b] | 74.5 ± 5.7[b] |
| GST | 179.6 ± 12.4 | 160.6 ± 6.8[a] | 170.1 ± 13.7 | 170.4 ± 11.7 | 172.67 ± 4.6[b] | 173.33 ± 12.4 |
| GSH | 29.1 ± 0.4 | 28.3 ± 0.2[a] | 30.9 ± 1.0[b] | 30.88 ± 2.1[b] | 30.80 ± 3.4 | 31.74 ± 2.0[b] |
| SOD | 6.8 ± 0.2 | 6.7 ± 0.1 | 7.81 ± 0.4[b] | 7.91 ± 0.2[b] | 8.28 ± 0.8[b] | 8.55 ± 0.5[b] |
| Total Thiol | 36.33 ± 3.7 | 18.5 ± 1.0[a] | 19.45 ± 0.4 | 20.07 ± 0.9[b] | 21.48 ± 1.6[b] | 19.78 ± 1.6 |

[a] Superscript (a) indicates significant decrease compared with Control at $P < 0.05$; Superscript (b) indicates significant increase compared with toxicant (lead exposed) groups at $P < 0.05$. GPx (Glutathione peroxidase, units/mg protein); GST (Glutathione-S-transferase; mmole/min/mg protein); GSH (Reduced Glutathione; micromole/g tissue); SOD (Superoxide dismutase; units/mg protein); TOTAL THIOL (nmole/mg protein).

**Table 3**
Effect of *Terminalia catappa* (TC), lisinopril and vitamin E on blood pressure parameters[a].

| | Parameters | Control | Lead | Lead + TC 100 mg/kg | Lead + TC 200 mg/kg | Lead + Vit.E | Lead + Lisinopril |
|---|---|---|---|---|---|---|---|
| Week 0 | Systolic BP mmHg | 126.0 ± 2.0 | 123.0 ± 2.1 | 125.1 ± 2.1 | 123.0 ± 1.8 | 125.0 ± 2.1 | 126.0 ± 2.0 |
| | Diastolic BP mmHg | 102.0 ± 1.7 | 101.0 ± 1.5 | 101.0 ± 1.3 | 99.0 ± 1.6 | 100.1 ± 1.8 | 101.1 ± 1.1 |
| | Mean BP mmHg | 113.0 ± 1.2 | 112.0 ± 1.0 | 112.0 ± 1.4 | 114.0 ± 1.2 | 110.1 ± 1.3 | 113.1 ± 1.4 |
| Week 12 | Systolic BP mmHg | 127.0 ± 2.5 | 180.5 ± 2.0 | 139.4 ± 2.2 [b] | 136.i ± 1.8 [b] | 146.2 ± 2.1 [b] | 146.4 ± 1.5[b] |
| | Diastolic BP mmHg | 103.0 ± 1.2 | 151.0 ± 2.3 | 126.3 ± 2. [b] | 115.0 ± 2.0[b] | 121.1 ± 1.8 [b] | 119.0 ± 2.1[b] |
| | Mean BP mmHg | 113.0 ± 2.6 | 166.2 ± 2.2 | 132.4 ± 1.6[b] | 131.8 ± 1.1 [b] | 130.0 ± 1.0[b] | 128.4 ± 1.2 [b] |

[a] Superscript (a) indicates significant decrease compared with Control at $P < 0.05$; Superscript (b) indicates significant increase compared with toxicant (lead exposed) groups at $P < 0.05$.

**Table 4**
Cardiac and renal absolute and relative organ weights (g).

| Parameters | Control | Lead | Lead + Extract 100 mg/kg | Lead + Extract 200 mg/kg | Lead + Vitamin E | Lead + Lisinopril |
|---|---|---|---|---|---|---|
| Heart | 0.51 ± 0.03 | 0.43 ± 0.02[a] | 0.39 ± 0.05[a] | 0.52 ± 0.02[a] | 0.49 ± 0.07[a] | 0.55 ± 0.04[a] |
| Heart/Body | 0.008 ± 0.004 | 0.007 ± 0.002[b] | 0.005 ± 0.003[b] | 0.005 ± 0.001[b] | 0.006 ± 0.002[b] | 0.007 ± 0.002[b] |
| Kidney | 0.65 ± 0.06[c] | 0.59 ± 0.20[c] | 0.80 ± 0.03[c] | 0.69 ± 0.04[c] | 0.71 ± 0.09[c] | 0.62 ± 0.03[c] |
| Kidney/Body | 0.004 ± 0.00[d] | 0.005 ± 0.002[d] | 0.006 ± 0.002[d] | 0.006 ± 0.003[d] | 0.005 ± 0.03[d] | 0.005 ± 0.002[d] |

[a] Superscript (a–d) indicate non-significant difference compared with Control at $P < 0.05$.

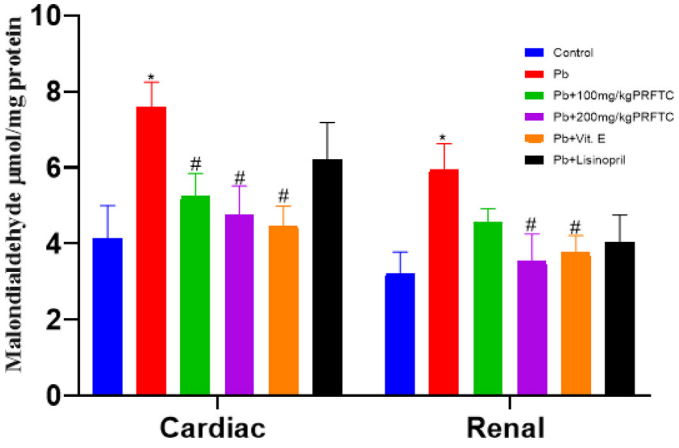


**Fig. 2.** Malondialdehyde in cardiac and Renal Tissues. Superscript (*) indicates significant increase compared with Control; Superscript (#) indicates significant decrease compared with Pb.

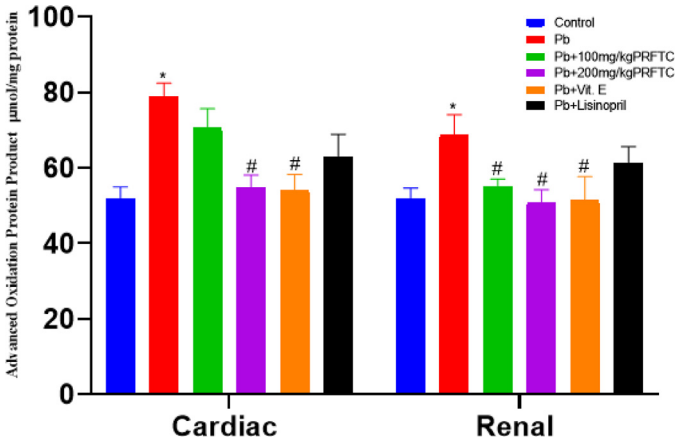


**Fig. 3.** Advanced oxidation protein product in cardiac and renal tissues. Malondialdehyde in cardiac and renal tissues. Superscript (*) indicates significant increase compared with Control; Superscript (#) indicates significant decrease compared with Pb.

Treatment with PRFTC and vitamin E significantly reduced serum MPO activity. Pb exposure caused a significant increase ($P < 0.05$) in the serum XO activity in comparison to groups treated with PRFTC, vitamin E and Lisinopril (Fig. 5). The Pb exposed rats had a significant decrease in the serum NO level, compared with the Control (Fig. 6). However, PRFTC and vitamin E significantly counteracted the lead acetate-induced reduction in NO level.

### 3.5. Effect of PRFTC on the histology of cardiac and renal tissues

The histopathology of the kidney showed that Pb exposure caused severe disseminated congestion of vessels and engorgement of the glomeruli with red blood cells (Fig. 7). In the heart, Pb exposure caused severe congestion of the cardiac cells (Fig. 8). However, the histopatho-

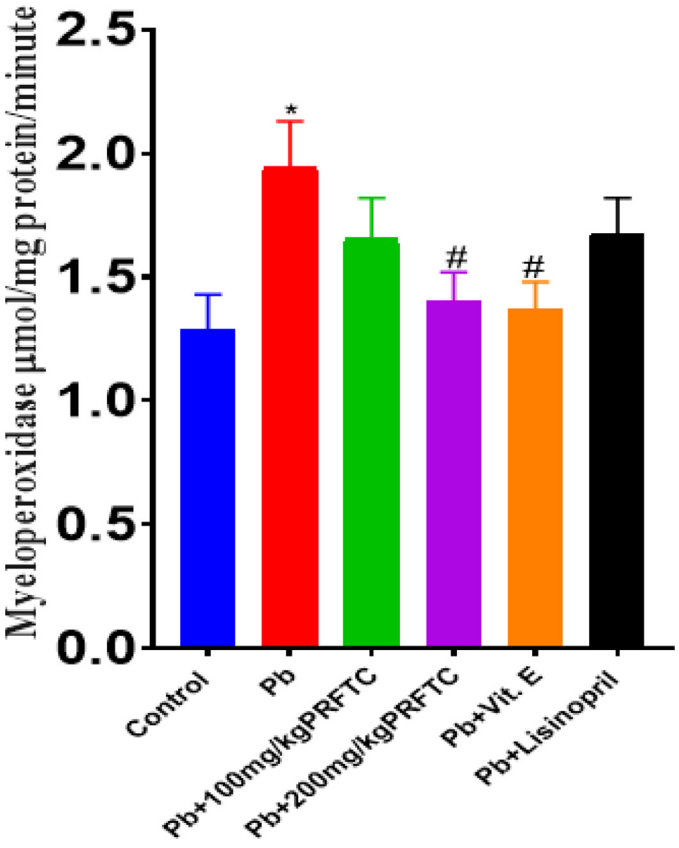


**Fig. 4.** Serum myeloperoxidase level. Superscript (*) indicates significant increase compared with Control; Superscript (#) indicates significant decrease compared with Pb.

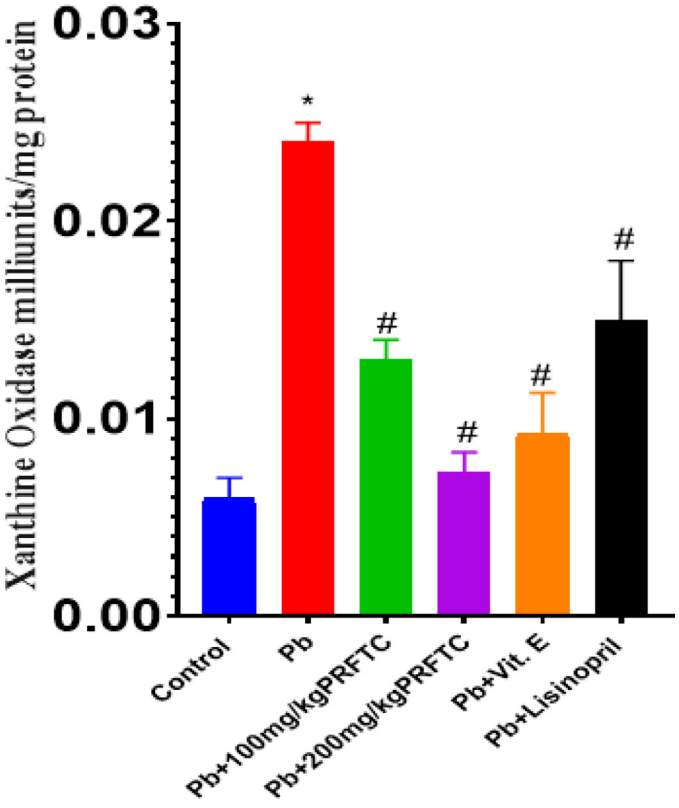


**Fig. 5.** Serum xanthine oxidase level. Superscript (*) indicates significant increase compared with Control; Superscript (#) indicates significant decrease compared with Pb.

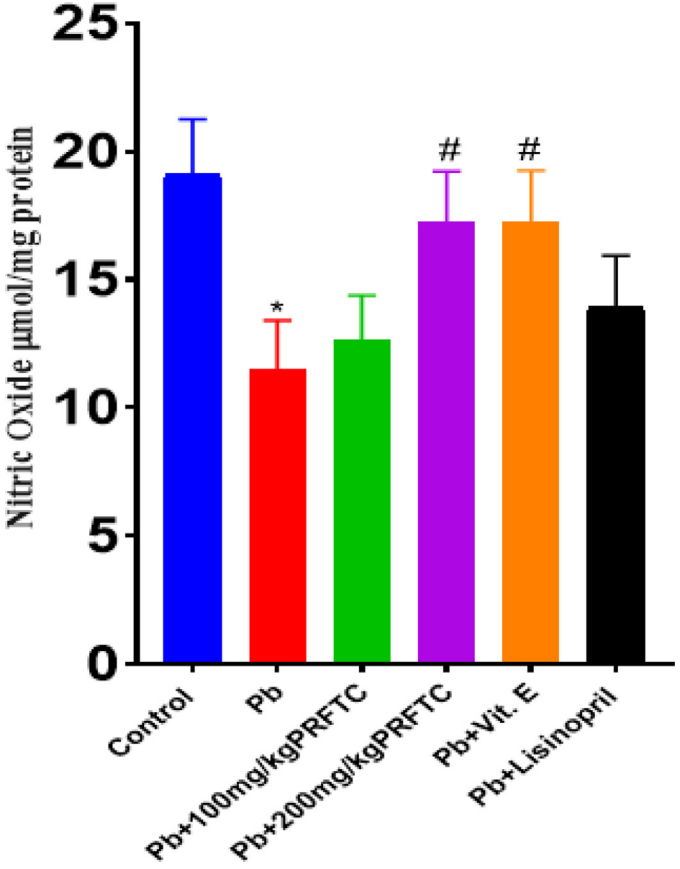


**Fig. 6.** Serum nitric oxide level. Superscript (*) indicates significant increase compared with Control; Superscript (#) indicates significant decrease compared with Pb.

logic lesions were mitigated in the rats treated with PRFTC and vitamin E.

### *3.6. Immunohistochemistry of cardiac and renal Caspase-3*

The immunohistochemical analysis revealed that the rats exposed to Pb showed higher immune-positive reactions to Caspase-3 in both renal and cardiac tissues than rats treated with PRFTC and vitamin E (Figs. 9 and 10).

## 4. Discussion

Oxidative stress has been well described as an integral component and one of the fundamental mechanisms implicated in the pathogenesis of the hypertensive state and several other toxicities associated with Pb intoxication (Tanito et al., 2004; Fioresi et al., 2014). Reactive oxygen species (ROS) play an important role in vascular homeostasis, and thus could contribute to the mechanism of hypertension (Lassègue and Griendling, 2004). Several studies have demonstrated that essential hypertensive patients and various animal models of hypertension produce excessive amounts of reactive oxygen species, and have abnormal antioxidant status; thereby contributing to accumulating evidence that increased vascular oxidative stress could be involved in the pathogenesis of essential hypertension (Paravicini and Touyz, 2006). Oxidative stress is associated with increased formation of reactive oxygen species that alter phospholipids and proteins, leading to peroxidation and oxidation of thiol groups (Molavi and Mehta, 2004). As a result, there is alterations in membrane permeability and functional modification of various cellular proteins in tissues and organs.

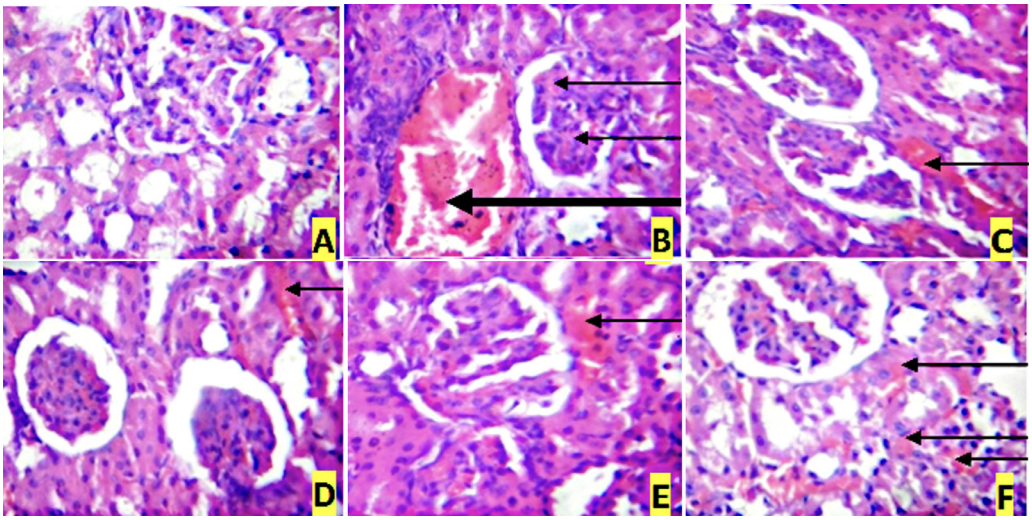


**Fig. 7.** Respective images of HE stained kidney tissue. Mag. X 400. A (Control); B (lead acetate, Pb) shows disseminated congestion of vessels as well as haemorrhagic lesion (thick arrow) and the glomeruli engorged with red blood cells (slender arrows); C (Pb, 100 PRFTC); D (200 PRFTC); E (Pb, vitamin E); F (Pb, lisinopril).

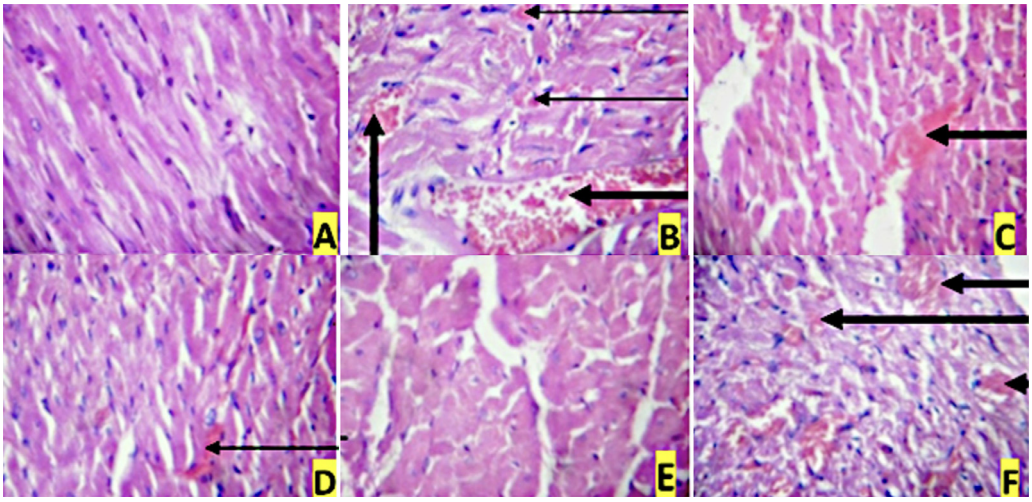


**Fig. 8.** Respective images of HE stained heart tissue. Mag. X 400. A (Control); B (lead acetate, Pb) shows severe congestion (thick arrow); C (Pb, 100 PRFTC); D (200 PRFTC); E (Pb, vitamin E); F (Pb, lisinopril).

In this study, the observed increase in the assayed markers of oxidative stress (MDA, MPO, AOPP, and XO) corroborates earlier reports implicating oxidative stress in the pathogenesis of lead acetate toxicity. Oxidative stress in tissue causes MDA, one of the final products of polyunsaturated fatty acids peroxidation in cells, to increase due to an elevation of free radicals (Gaweł et al., 2004). Also, MPO which is an important enzyme in the generation of reactive oxygen radicals (Ho et al., 2000) observed to be elevated in this study promotes endothelial dysfunction by the conversion of hydrogen peroxide to various reactive oxygen species including ·OH, ONOO- and hypochlorous acid (HOCl) (Abu-Soud et al., 2000). These reactive oxygen species can then modify lipids, lipoproteins and proteins, and cause deleterious alterations in biological system. In this study, significant increases were observed in serum myeloperoxidase levels in the lead acetate exposed group when compared with control, but the administration of the polyphenolic fraction of *T. catappa* significantly reduced the MPO level. Furthermore, increased plasma level of AOPP, as observed in this study, suggests an ability of Pb to induce oxidative stress in the cardiovascular system, thereby triggering hypertension and its associated complications. In a study carried out by Gonzalez et al. (Gonzalez et al., 2014), it was reported that plasma AOPP levels increase over time; especially among patients with cardiovascular diseases and also in patients with subsequent cardiovascular events suggesting that the change in AOPP level may be considered a marker of a permanent cardiovascular risk status. Xanthine oxidase (XO) typically catalyses the oxidation of hypoxanthine to xanthine and subsequent oxidation of xanthine to uric acid (Cao et al., 2014). However, the activity of XO increases the formation of $O_2^-$ in the vascular endothelium, thereby inducing oxidative stress and increasing arteriolar tone (Witko-Sarsat et al., 1998).

Pb exposure has been reported to increase the generation of superoxide and hydrogen peroxide in endothelial and vascular smooth muscle cells (Sebeková et al., 2012). Hydrogen peroxide is decomposed to $H_2O$ and $O_2$ by a reaction catalysed mainly by catalase (CAT) and glutathione peroxidise (GPx) (Lacy et al., 1998). The reduction in the activity of CAT and GPx, as observed in this study, suggests an aggravation of oxidative stress and may be responsible for the severe hypertension recorded in the Pb exposed rats. Reduced GPx activity reportedly leads to an increased $H_2O_2$ with subsequent tissue damage and reduction in nitric oxide (NO) level, heightened lipid peroxidation, and impaired endothelial function are well reported features of Pb-induced hypertension (Suzuki et al., 1998).

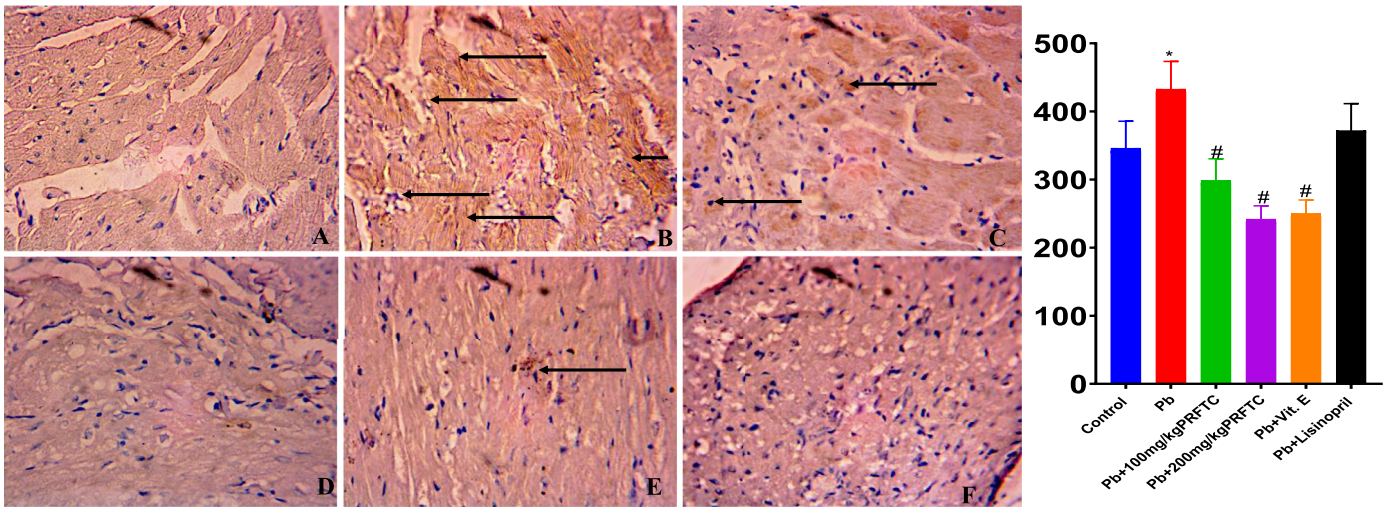


**Fig. 9.** Immunohistochemistry of Caspase-3 in the cardiac tissue of rats. Mag.x100. A (Control); B (lead acetate, Pb) shows high expression of Caspase-3; C (Pb, 100 PRFTC); D (200 PRFTC) shows low expression of Caspase-3; E (Pb, vitamin E); F (Pb, lisinopril). Superscript (*) indicates significant increase compared with Control; Superscript (#) indicates significant decrease compared with Pb.

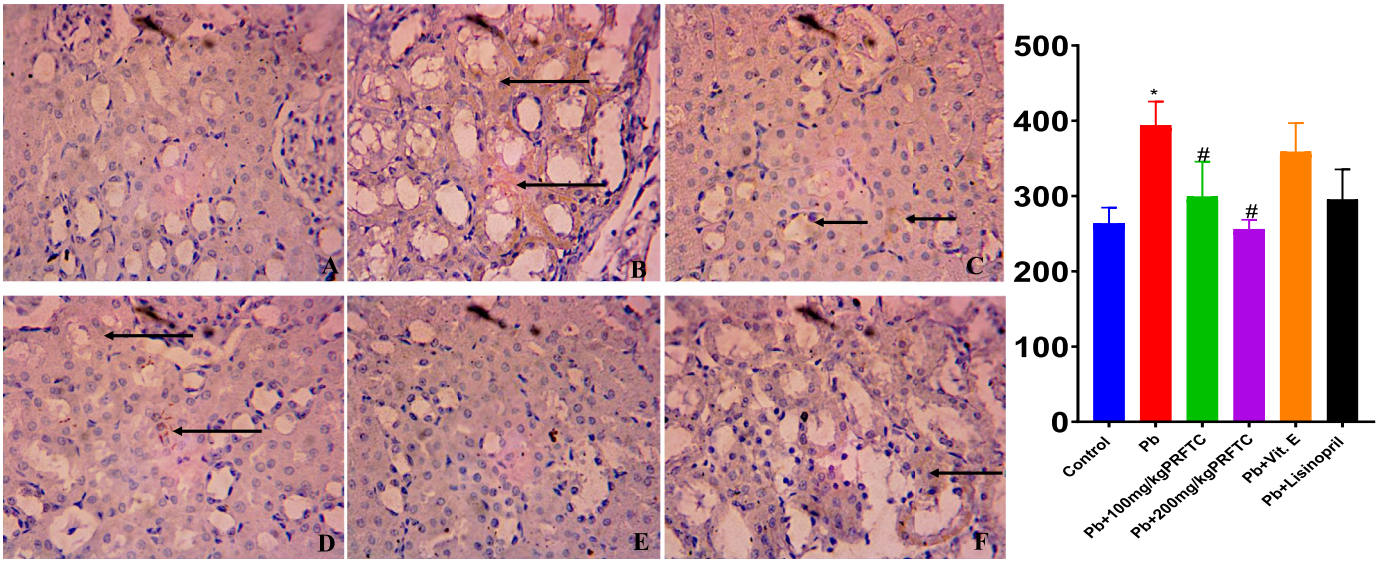


**Fig. 10.** Immunohistochemistry of Caspase-3 in the renal tissue of rats. Mag.x100. A (Control); B (lead acetate, Pb) shows high expression of caspase 3; C (Pb, 100 PRFTC); D (200 PRFTC) shows low expression of caspase 3; E (Pb, vitamin E); F (Pb, lisinopril). Superscript (*) indicates significant increase compared with Control; Superscript (#) indicates significant decrease compared with Pb.

Endothelial cells play a major role in the control of vascular tone. These endothelial cells produce NO which is important for vascular relaxation. Nitric oxide is rapidly degraded by the oxygen-derived free radical superoxide anion. Superoxide anion acts as a vasoconstrictor and influences the biosynthesis and bioavailability of nitric oxide. Thus, it can modify the vascular endothelial function. The observation of significant reduction in the blood pressure parameters of rats treated with PRFTC suggests a protective role for the extract in oxidative stress-mediated Pb-induced hypertension. Interestingly, the histopathologic lesions and expression of Caspase-3, the rate limiting molecule in the induction of apoptosis, in the cardiac and renal tissues were less severe in PRFTC treated rats. Caspase-3 reportedly mediates the downstream component of the mitochondrial pathway-mediated cell death, after dysfunction of the mitochondria and the release of cytochrome c into the cytoplasm, with several reports implicating the modulation of Caspase-3 expression in Pb-induced apoptosis in the reproductive system, the liver, kidney, and human leukemia (HL-60) cells, with Caspase-3 probably executing apoptotic DNA damage (Elgawish and Abdelrazek, 2014). Moreover, apoptosis, which has been reported as one of the pathogenic events of Pb induced renal damage, is a defining pathogenic event in many cardiovascular disorders including heart failure (Liu et al., 2000). The toxicological effects of Pb on renal tissue is often associated with its prooxidative effects resulting from the induction of excessive reactive oxygen species formation and consequent apoptotic cellular damage (Abdel-Moneim et al., 2011). Therefore, the observation of decreased expression of Caspase-3 in the renal and cardiac tissues of rats treated with PRFTC, in this study, suggests mitigation of apoptotic processes as corroborated by earlier reports of Rong et al. (Rong et al., 2008), who suggested that the toxic effects of Pb on the kidney is probably induced by an induction of accelerated apoptotic mechanisms associated with direct activation of NF-κB and p53 genes, with concurrent attenuation of the *Bcl2* gene. Moreover, it is probable that PRFTC, due to its high polyphnolic properties usually attributable to flavonoids that act as metal chelators due to the presence of multiple hydroxyl groups forming

a coordination bond with Pb, exerts a potent chelating effect or is able to increase the rate of removal of Pb from the kidney thereby reducing its renal toxicity (Dkhil et al., 2016).

## 5. Conclusion

The polyphenol-rich fraction of *T. catappa* proved effective in the reduction of oxidative stress-mediated derangements of the physiological homeostasis and decreased apoptosis in the cardiovascular and renal systems of rats chronically exposed to lead acetate toxicities and may therefore have therapeutic potential as a supplement that can be applied in chronic lead acetate poisoning.

## Ethical Approval

The ethical approval for this study was obtained from the University of Ibadan Animal Care Research Ethics Committee of the Faculty of Veterinary Medicine University of Ibadan (Approval Number: UI-ACUREC/17/065).

## Data Availability

Data for this study are available if required.

## Funding

Nil.

## Declaration of Competing Interest

The authors have no conflicting interest to declare.

## CRediT authorship contribution statement

**Temitayo Ajibade:** Conceptualization, Validation, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Adedeji Adebayo:** Investigation, Resources, Investigation, Project administration. **Ademola Oyagbemi:** Resources, Writing – review & editing, Data curation, Supervision. **Temidayo Omobowale:** Resources, Supervision.

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## Supplementary Materials

Nil.

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