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# Ameliorating effect of ethanolic leaf extract of T*elfairia occidentalis* on the histology of livers of lead-induced rats

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## Abstract

**Objective:** This research study was carried out to investigate the ameliorating effect of ethanolic leaf extract of *Telfairia occendentalis* on the histology of livers of lead-induced rats.

**Methodology:** Twenty-five (25) male wistar rats weighing 150 - 180 g were procured and acclimatized for two weeks, after which, they were divided into five (5) groups of five (5) rats each, and were housed in cages. The groups were designated as groups A - E. Group A served as the control group and was not induced with lead (Pb), while Groups B – E were induced. Group A received distilled water only, Groups B - E received vitamin C, vitamin C + 100 mg/kg of ethanolic leaf extract *T. occendentalis,* vitamin C + 400 mg/kg ethanolic leaf extract of *T. occendentalis* and vitamin C + 800 mg/kg ethanolic leaf extract of *T. occendentalis* respectively for 14 days through oral route with the aid of oral gastric tube. On the 15th day, the animals were weighed and sacrificed via chloroform inhalation, and kidneys were harvested from the rats for histological study.

**Results:** Histopathological findings showed normal liver architecture with well perfused normal hepatic architecture with central vein (CV), hepatocyte (H) and portal traid (PT) for animals in group A; moderate degeneration with moderate congestion of blood vessel (CBV) with aggregate inflammatory cell (AIC) around the congested vessel and intra hepatic inflammation (IHI) for animals in group B; mild regeneration with moderate congestion of blood vessel (CBV) with moderate intra hepatic hemorrhage (IHH) and intra hepatic inflammation (IHI) for animals in group C; moderate regeneration with mild congestion of portal vain (CPV) with portal aggregate inflammatory cell (AIC) and mild intra hepatic inflammation (IHI) for animals in group D; and moderate regeneration with mild congestion of portal vain (CPV) with portal aggregate inflammatory cell (PAIC) for animals in group E.

**Conclusion:** Ethanolic leaf extract of *T. occendentalis* have ameliorating effect on the histology of Livers of lead-induced rats, and the ameliorating effect improves with increase in the dosages of the leaf extract.

**Keywords:** *Telfairia occendentalis*; Liver; Lead; Vitamin C.

## 1. Introduction

Lead is a chemical element with the symbol Pb and atomic number 82 [1]. It is a heavy metal that is denser than most common materials. It is soft and malleable, has relatively low melting point, and its atom has 82 electrons, arranged in an electron configuration of $[Xe]4f^{14}5d^{10}6s^{2}6p^{2}$ [1]. It has no confirmed biological role, and there is no confirmed safe

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level of its exposure [2]. According to Bouchard *et al.,* [3] even at levels that are considered to pose little to no risk, it may cause "adverse mental health outcomes". Its prevalence in the human body — at an adult average of 120 mg [4] — is nevertheless exceeded only by zinc (2500 mg) and iron (4000 mg) among the heavy metals [5]. Its salts are very efficiently absorbed by the body [6], and a small amount of it (1 %) is stored in bones; the rest is excreted in urine and feces within a few weeks of exposure. Only about a third of it is excreted by a child, and continual exposure may result in its bioaccumulation [7]. Its exposure is a global issue since its mining and smelting, and battery manufacturing, disposal, and recycling, are common in many countries. It enters the body via inhalation, ingestion, or skin absorption. Almost all inhaled lead is absorbed into the body; for ingestion, the rate is 20–70 %, with children absorbing a higher percentage than adults [8]. Elevated concentrations of lead persist in soils and sediments in post-industrial and urban areas; industrial emissions, including those arising from coal burning [9] continue in many parts of the world, particularly in the developing countries [10]. It can accumulate in soils, especially those with a high organic content, where it remains for hundreds to thousands of years [1].

Environmental lead can compete with other metals found in and on plant surfaces potentially inhibiting photosynthesis and at high enough concentrations, negatively affecting plant growth and survival [1]. Contamination of soils and plants can allow lead to ascend the food chain affecting microorganisms and animals. In animals, lead exhibits toxicity in many organs, damaging the nervous, renal, reproductive, hematopoietic, and cardiovascular systems after ingestion, inhalation, or skin absorption [11]. Fish uptake lead from both water and sediment [12]; bioaccumulation in the food chain poses a hazard to fish, birds, and sea mammals [13]. It is a highly poisonous metal (whether inhaled or swallowed), affecting almost every organ and system in the human body [14]. At airborne levels of 100 mg/m$^3$, it is immediately dangerous to life and health [15]. Most ingested lead is absorbed into the bloodstream [18]. The primary cause of its toxicity is its predilection for interfering with the proper functioning of enzymes. It does so by binding to the sulfhydryl groups found on many enzymes [17] or mimicking and displacing other metals which act as cofactors in many enzymatic reactions Dart *et al.,* [18]. The essential metals that lead interacts with include calcium, iron, and zinc [19]. High levels of calcium and iron tend to provide some protection from lead poisoning; low levels cause increased susceptibility [20].

It can cause severe damage to the brain and kidneys and, ultimately, death. By mimicking calcium, lead can cross the blood–brain barrier. It degrades the myelin sheaths of neurons, reduces their numbers, interferes with neurotransmission routes, and decreases neuronal growth [17]. In the human body, it inhibits porphobilinogen synthase and ferrochelatase, preventing both porphobilinogen formation and the incorporation of iron into protoporphyrin IX, the final step in heme synthesis. This causes ineffective heme synthesis and microcytic anemia [21]. In a child's developing brain, lead interferes with synapse formation in the cerebral cortex, neurochemical development (including that of neurotransmitters), and the organization of ion channels [22]. Early childhood exposure has been linked with an increased risk of sleep disturbances and excessive daytime drowsiness in later childhood [23]. Its high blood levels are associated with delayed puberty in girls [24]. According to Prasad [25] treatment for lead poisoning normally involves the administration of dimercaprol and succimer. Acute cases may require the use of disodium calcium edetate, the calcium chelate, and the disodium salt of ethylenediaminetetraacetic acid (EDTA). It has a greater affinity for lead than calcium, with the result that lead chelate is formed by exchange and excreted in the urine, leaving behind harmless calcium [26].

The liver is a dark reddish brown, wedge-shaped organ with two lobes of unequal size and shape [27]. It is both the heaviest internal organ and the largest gland in the human body; and is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm, to the right of the stomach, and overlying the gallbladder [28]. It a major metabolic organ only found in vertebrate animals, which performs many essential biological functions such as detoxification of the organism, and the synthesis of proteins and biochemicals necessary for digestion and growth [29; 30]. It is also an accessory organ of digestion that produces bile, an alkaline fluid containing cholesterol and bile acids, which emulsifies and aids the breakdown of dietary fat. Its highly specialized tissue, consisting mostly of hepatocytes, regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex organic molecules, many of which are necessary for normal vital functions [31].

The liver is connected to two large blood vessels: the hepatic artery and the portal vein. The hepatic artery carries oxygen-rich blood from the aorta via the celiac trunk, whereas the portal vein carries blood rich in digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas [32]. These blood vessels subdivide into small capillaries known as liver sinusoids, which then lead to hepatic lobules - the functional units of the liver. Each lobule is made up of millions of hepatic cells (hepatocytes), which are the basic metabolic cells. The lobules are held together by a fine, dense, irregular, fibroelastic connective tissue layer extending from the fibrous capsule covering the entire liver known as *Glisson's capsule* after British doctor Francis Glisson [33]. This tissue extends into the structure of the liver by accompanying the blood vessels, ducts, and nerves at the hepatic hilum. The whole surface of the liver,

except for the bare area, is covered in a serous coat derived from the peritoneum, and this firmly adheres to the inner Glisson's capsule [27].

Microscopically, each liver lobe is seen to be made up of hepatic lobules. The lobules are roughly hexagonal, and consist of plates of hepatocytes, and sinusoids radiating from a central vein towards an imaginary perimeter of interlobular portal triads [34]. The central vein joins to the hepatic vein to carry blood out from the liver. A distinctive component of a lobule is the portal triad, which can be found running along each of the lobule's corners. The portal triad consists of the hepatic artery, the portal vein, and the common bile duct [34]. The triad may be seen on a liver ultrasound, as a Mickey Mouse sign with the portal vein as the head, and the hepatic artery, and the common bile duct as the ears [34]. The study of its microscopic anatomy shows two major types of liver cell: parenchymal cells and nonparenchymal cells. About 70–85 % of the liver volume is occupied by parenchymal hepatocytes. Nonparenchymal cells constitute 40% of the total number of liver cells but only 6.5 % of its volume [35]. The liver sinusoids are lined with two types of cell, sinusoidal endothelial cells, and phagocytic Kupffer cells [36]. Hepatic stellate cells are nonparenchymal cells found in the perisinusoidal space, between a sinusoid and a hepatocyte [35]. Additionally, intrahepatic lymphocytes are often present in the sinusoidal lumen [35].

*Telfairia occidentalis* is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds [37]. Its common names include fluted gourd, fluted pumpkin, ugu (in the Igbo language), okwukwo-wiri (in Ikwerre language), ikong-ubong (in the Efik and Ibibio languages), and "Akwukwor ri" (in Etche language)' [37]. *'T. occidentalis* is a member of the family *Cucurbitaceae* and is indigenous to southern Nigeria [38]. It is traditionally used by an estimated 30 to 35 million people in Nigeria, including the Efik, Ibibio, Ikwerre, and Urhobo ethnic groups [38]. However, it is predominantly used by the Igbo ethnic group, who continue to cultivate the gourd for food sources and traditional medicines [39]. A recurring subject in the Igbo's folklore, the fluted gourd is noted to have healing properties and was used as a blood tonic, to be administered to the weak or ill [38]. It is endemic to southern Nigeria, and was an asset to international food trades of the Igbo ethnic group [38]. Its fruit is quite large; one study documented a range of 16–105 cm (6.3–41.3 in) in length, and an average of 9 cm in diameter [39]. The seed count in larger fluted gourds fruits can reach upwards of 196 per fruit, typically measuring between 3.4 and 4.9 cm in length [39]. In both the pistillate and staminate varieties, *T. occidentalis* flowers grow in sets of five, with creamy-white and dark red petals, contrasting with the light green colour of the fruit when young, and yellow when ripe [39]. Dioecious flowering is most common in the fluted gourd, with very few documented cases of monoecious flowering [37].

*T. occidentalis*' fluted gourd is high in oil (30 %) [38], its shoots contain high levels of potassium and iron, while its seeds are composed of 27 % crude proteins and 53 % fats [40]. Its leaves contain a high number of antioxidants, hepatoprotective and antimicrobial properties [41]. While the young shoots and leaves of the *T. occidentali* female plant are the main ingredients of a Nigerian soup, *ofe egwusi*. Its large (up to 5 cm), dark-red seed is rich in fat and protein, and can be eaten whole, ground into powder for a kind of soup, or made into a fermented porridge (https3). *T. occidentalis*' edible seeds can be boiled and eaten whole, or fermented and added to *ogili* [42]. The fluted gourd has been traditionally used by indigenous tribes as a blood tonic, likely due to its high protein content [38]. Flour produced from the seeds can be used for high-protein breads. Furthermore, the shoots and leaves can be consumed as vegetables [38]. When *T. occidentalis* is prepared for herbal medicine, it is used to treat sudden attack of convulsion, malaria, and anaemia; it also plays a vital and protective role in cardiovascular diseases [43].

*T. occidentalis* leaf enhances blood production, serve as excellent source of dietary fiber, function as anti-diabetic agent, protects body's tissues, and strengthens bones teeth (https4). Other benefits include treatment of convulsion, antioxidant-rich, hormone harmonization, weight loss effective, helps fertility, lactating characteristics, anti-inflammatory effects, antimicrobial properties, treatment of anaemia, lowers the risk of kidney disease, helps to prevent cancer, antioxidant abilities, stress relief, prevents Alzheimer's disease and dementia, treats infertility and immune system booster [37]. Also, its leaf extract might lead to membrane stabilizing effects on hepatocytes, depressed hepatocyte synthetic activity and impaired renal function, and has effectively maintain electrolyte balance, modulates pancytopenia and oxidative renal damage [39] in rats suggesting its protective potentials on anaemia and renal disorders [45]. It is also a better blood boosting vegetable than *A. conyzoides* [46]. Lastly, research has shown that the leaf extract of *T. occendentalis* has ameliorating effect on the histology of kidneys of lead-induced wistar rats, and the ameliorating effect improves with increase in the dosages of the leaf extract [47].

Therefore, this study will help to create more awareness on the ameliorating effect of the leaf extract of *T. occidentalis* on lead related poisoning thereby encourage regular consumption of the leaf to reduce effects of lead poisoning which affects most of the body organs.

## 2. Materials and methods

### 2.1. Animal procurement, care, and treatment

Fwenty-five (25) male wistar rats weighing between 150 – 180 g were procured and housed at the Animal house of Anatomy Department, Abia State University; Uturu with wire gauze cages in a well-ventilated area, were maintained under standard laboratory conditions of temperature (22+2 $^0$C), relative humidity (55-65 %) and 12 hours light/dark cycle [47]. They were fed with standard commercial pellet diet and water *ad libitum* and were also acclimatized for two weeks before the experiment. Their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

### 2.2. Collection, identification, and preparation of plant material

Fresh leaves of *T. occidentalis* were purchased from a local market in Okigwe L.G.A., Imo State, and were authenticated at Herbarium unit, Botany Department, Abia State University, Uturu, Abia State. The leaves were air dried and crushed using laboratory blender. Extractions were done using ethanol. The crude ethanol extracts were kept in an air-tight container and stored in a refrigerator at 4 $^0$C until time of use. At the time of use, the ethanol extracts were filtered into a stainless basin with a white cloth and placed in a water bath to dry up the ethanol. 250 mg of these extracts /kg body weights were dissolved in 10 m/s of distilled water and were administered to the animals [47].

### 2.3. Induction of lead and Vitamin C administration

According to research study, the oral $LD_{50}$ of lead acetate has been calculated to be 600 mg/kg body weight for Wistar rats [48], thus, 5 % (30 mg/kg) of the lead acetate were used to induced the rats daily for fourteen (14) days. Also, 40 mg/kg body weight of vitamin C was administered to the lead-induced animals for the same number of days [47].

### 2.4. Experimental protocol

The animals were grouped into five (5) groups of five (5) rats each. Different doses of the leaf extracts were administered via oral route with the aid of oral gastric tube as shown below:

**Group A**: The control group + distilled water.

**Group B**: Lead + Vitamin C

**Group C**: Lead + Vitamin C + 100 mg/kg of *T. occidentalis* leaf extract.

**Group D**: Lead + Vitamin C + 400 mg/kg of *T, occidentalis* leaf extract.

**Group E**: Lead + Vitamin C + 800 mg/kg of *T. occidentalis* leaf extract. [47].

### 2.5. Sample collection and analysis

The extracts were administered for fourteen (14) days. On the 15$^{th}$ day, the animals were sacrificed by anaestethizing under chloroform vapour and dissected. Livers harvested from the rats were weighed, and fixed in Bouin's fluid for 72 hours, after which they were transferred to 10 % buffered formalin. This was followed by histological and histochemical methods of tissue processing.

## 3. Results

### 3.1. Histopathological findings

Results of the histopathological findings of the the ameliorating effect of ethanolic leaf extract of *Telfairia occendentalis* on the histology of livers of lead-induced rats are as shown below:

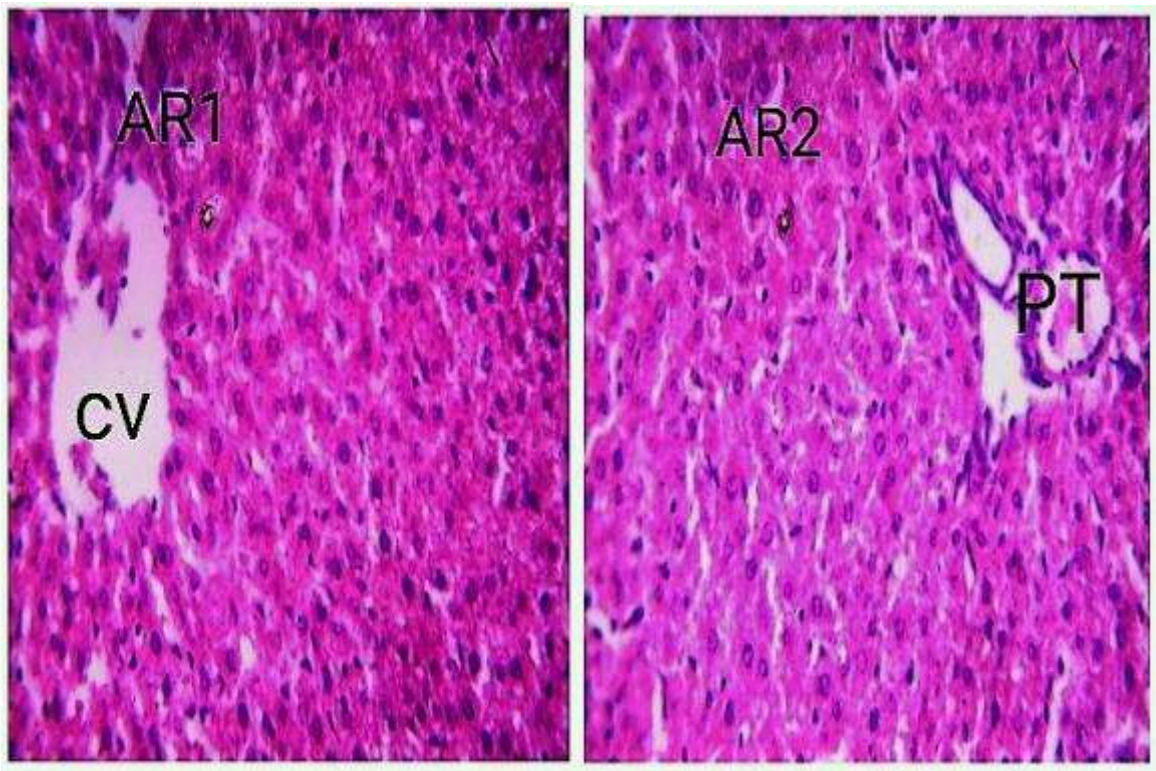


**Figure 1** This a photomicrograph of group A (R1R2) control section of liver (x400) (H/E) showing well perfused normal hepatic architecture with central vein (CV), hepatocyte (H) and portal traid (PT).

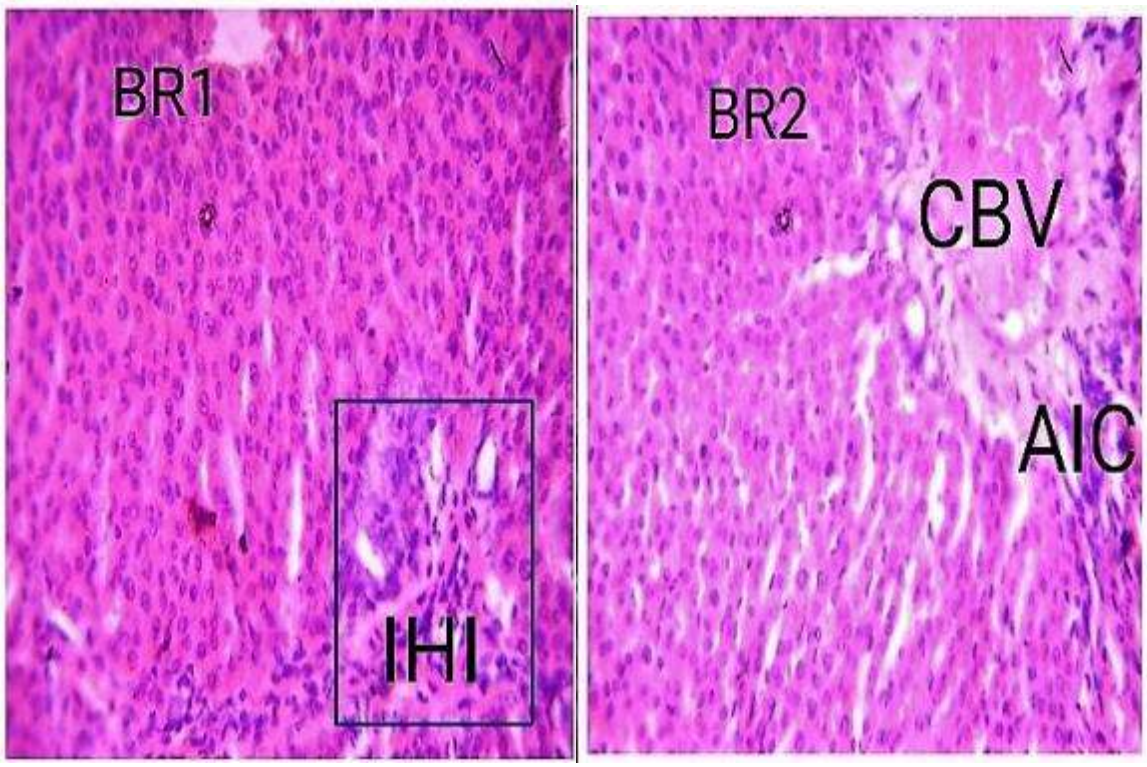


**Figure 2** This is a photomicrograph of group B (R1R2) section of liver induced with lead (x400) (H/E) showing moderate degeneration with moderate congestion of blood vessel (CBV) with aggregate inflammatory cell (AIC) around the congested vessel and intra hepatic inflammation (IHI).

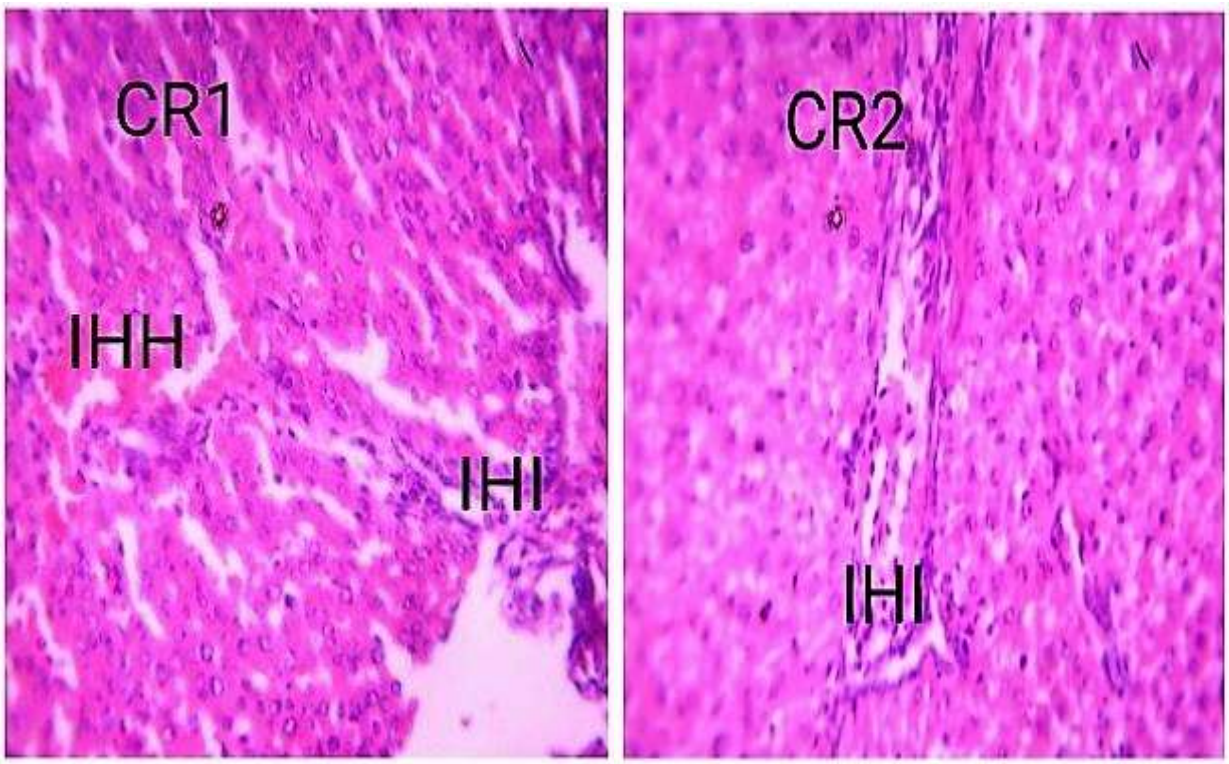


**Figure 3** This a photomicrograph of group C (R1R2) section of liver induced with lead and treated with 100 mg/kg of *T. occidentalis* leaf extract (x400) (H/E) showing mild regeneration with moderate congestion of blood vessel (CBV) with moderate intra hepatic hemorrhage (IHH) and intra hepatic inflammation (IHI).

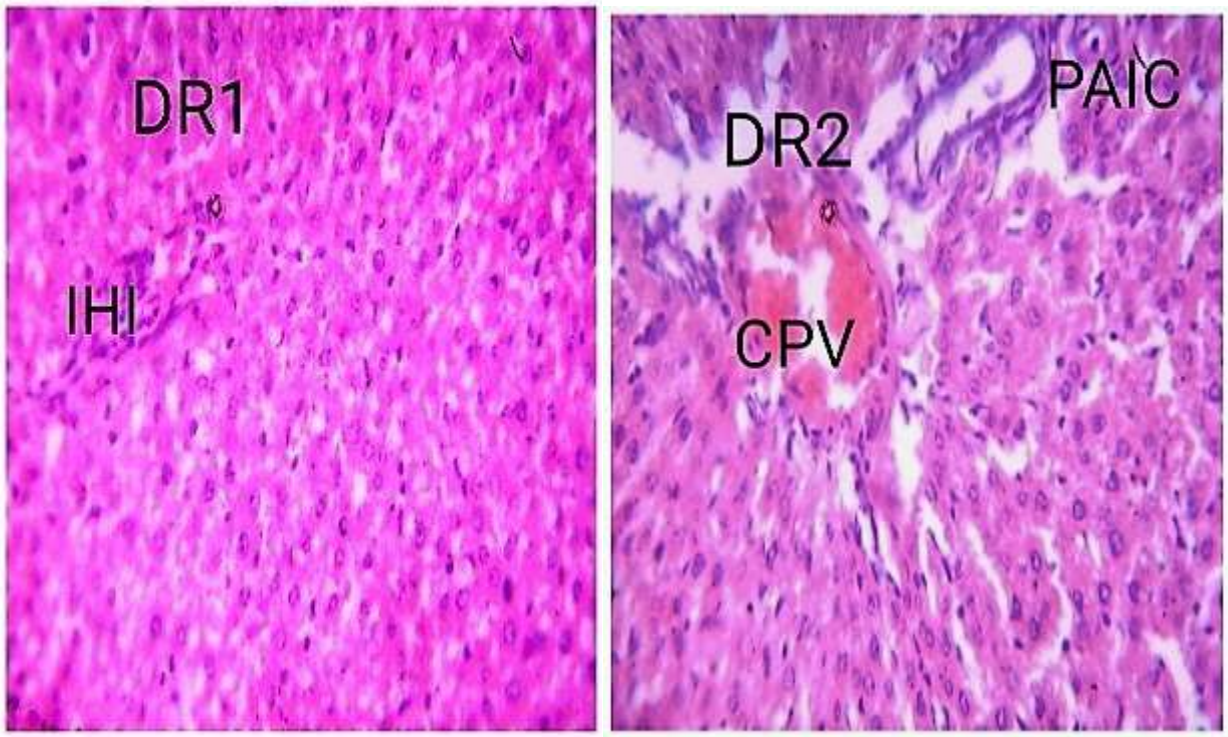


**Figure 4** This is a photomicrograph of group D (R1R2) section of liver induced with lead and treated with 400 mg/kg of *T. occidentalis* leaf extract (x400) (H/E) showing moderate regeneration with mild congestion of portal vain (CPV) with portal aggregate inflammatory cell (AIC) and mild intra hepatic inflammation (IHI).

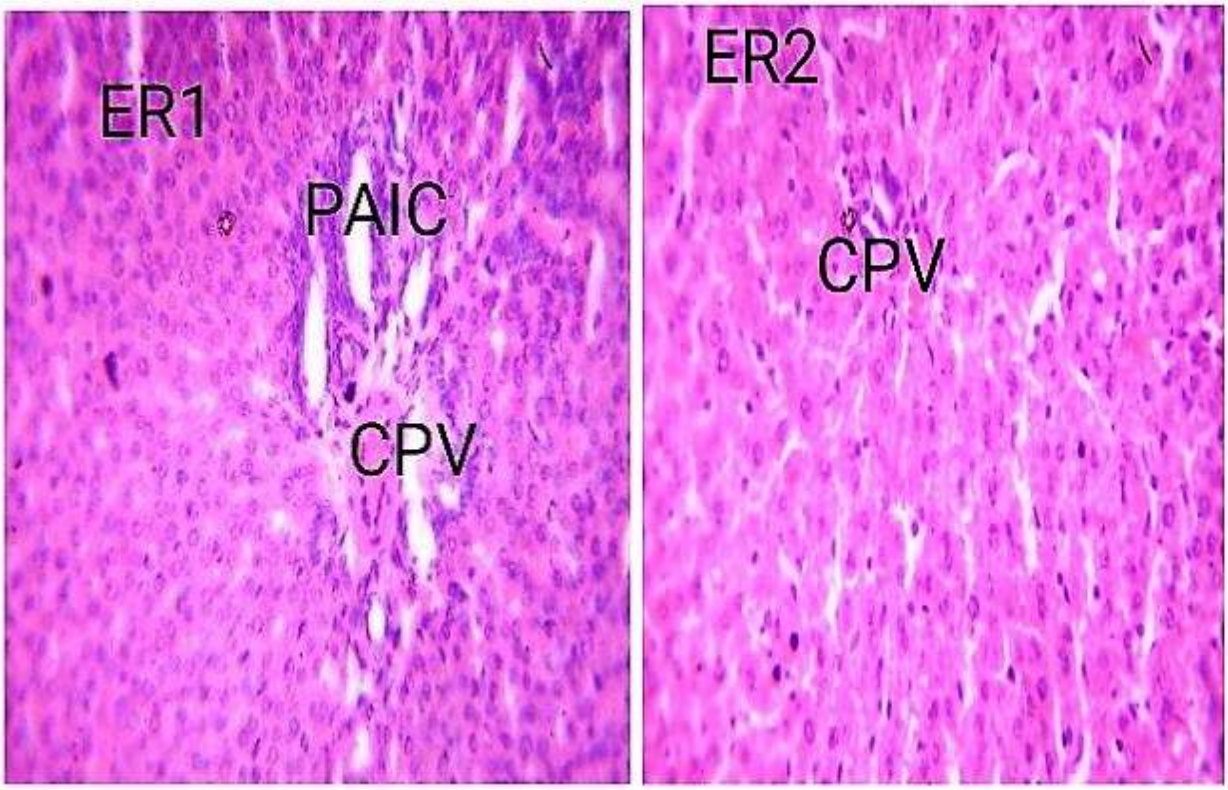


**Figure 5** This is a photomicrograph of group E (R1R2) section of liver induced with lead and treated with 800 mg/kg of *T. occidentalis* leaf extract (x400) (H/E) showing moderate regeneration with mild congestion of portal vain (CPV) with portal aggregate inflammatory cell (PAIC).

## 4. Discussion

Lead exposure is a global issue since its mining and smelting, and battery manufacturing, disposal, and recycling, are common in many countries. It enters the body via inhalation, ingestion, or skin absorption. Environmental lead can compete with other metals found in and on plant surfaces potentially inhibiting photosynthesis and at high enough concentrations, negatively affecting plant growth and survival [1]. Contamination of soils and plants can allow lead to ascend the food chain affecting microorganisms and animals. In animals, lead exhibits toxicity in many organs, damaging the nervous, renal, reproductive, hematopoietic, and cardiovascular systems after ingestion, inhalation, or skin absorption [11]. Fish uptake lead from both water and sediment [12]; bioaccumulation in the food chain poses a hazard to fish, birds, and sea mammals [13]. Lead is a highly poisonous metal (whether inhaled or swallowed), affecting almost every organ and system in the human body [14].

The histopathological finding of this present research study of figure 1 is the photomicrograph of group A (R1R2) control section of liver (x400) (H/E) showed normal liver architecture with well perfused normal hepatic architecture with central vein (CV), hepatocyte (H) and portal traid (PT) could be due to the none exposure of the rats to lead. While, the histopathological result of the histology of the livers of the animals in figure 2 is the photomicrograph of group B (R1R2) section of liver induced with lead (x400) (H/E) which showed moderate degeneration with moderate congestion of blood vessel (CBV) with aggregate inflammatory cell (AIC) around the congested vessel and intra hepatic inflammation (IHI), could be due to the toxic effect of the lead induced to the rats. Research has shown that in animals, lead exhibits toxicity in many organs, damaging the nervous, renal, reproductive, hematopoietic, and cardiovascular systems after ingestion, inhalation, or skin absorption [11]. The primary cause of its toxicity is its predilection for interfering with the proper functioning of enzymes. It does so by binding to the sulfhydryl groups found on many enzymes or mimicking and displacing other metals which act as cofactors in many enzymatic reactions [17]. Also in the human body, lead inhibits porphobilinogen synthase and ferrochelatase, preventing both porphobilinogen formation and the incorporation of iron into protoporphyrin IX, the final step in heme synthesis, thereby causes ineffective heme synthesis and microcytic anemia [21].

Figures 3, 4 and 5 of the photomicrographs of the livers of the animals in groups C, D, and E that were induced with lead and were given Vitamin C + 100 mg/kg, Vitamin C + 400 mg/kg, and Vitamin C + 800 mg/kg of *T. occidentalis* leaf extracts which showed mild regeneration with moderate congestion of blood vessel (CBV) with moderate intra hepatic hemorrhage (IHH) and intra hepatic inflammation (IHI); moderate regeneration with mild congestion of portal vain (CPV) with portal aggregate inflammatory cell (AIC) and mild intra hepatic inflammation (IHI); and moderate regeneration with mild congestion of portal vain (CPV) with portal aggregate inflammatory cell (PAIC) respectively could be due to ameliorating effect of the *T. occidentalis.* leaf extracts to the livers of the lead-induced rats. According to Okoye *et al,* [44], *T. occidentalis* leaf extract might lead to membrane stabilizing effects on hepatocytes, depressed hepatocyte synthetic activity and impaired renal function, and has effectively maintain electrolyte balance, modulates pancytopenia and oxidative renal damage in rats suggesting its protective potentials on anaemia and renal disorders [45].

Research has equally showed that *T. occendentalis* leaf enhances blood production, serve as excellent source of dietary fiber, function as anti-diabetic agent, protects body's tissues, and strengthens bones teeth, useful for the treatment of convulsion, antioxidant-rich, hormone harmonization, weight loss effective, helps fertility, lactating characteristics, anti-inflammatory effects, antimicrobial properties, treatment of anaemia, lowers the risk of kidney disease, helps to prevent cancer, antioxidant abilities, stress relief, prevents Alzheimer's disease and dementia, treats infertility and immune system booster [37]. It is also a better blood boosting vegetable than *A. conyzoides* [46]. Lastly, it has also be proven that the leaf extract of *T. occendentalis* has ameliorating effect on the histology of kidneys of lead-induced wistar rats, and the ameliorating effect improves with increase in the dosages of the leaf extract [47] as could be evidenced in this present study.

## 5. Conclusion

Leaf extract of *Telfairia occendentalis* has ameliorating effect on the histology of liver of lead-induced rats, and the ameliorating effect is dose-dependent, and improves better with increase in dosages of the leaf extract.

## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

There was no conflict of interest.

### *Statement of ethical approval*

This research work was approved by the Ethical Approval Committee, Human Anatomy Department, Abia State University, Uturu

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