Research Study

Nephro-toxicity and hepato-toxicity effects of lead acetate on rats used to model the dangerous acute environmental lead and the role of herbal products in restoring their histoarchitectural integrity

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The longer we live, the more our natural defense mechanism is depleted. These defense mechanisms are coordinated by both the liver and kidney. The liver and kidneys functions to detoxify and thermo-regulate the body too. **Objective:** The current study is designed to prove if the liver and kidney can either be protected or cured from the effects of the accumulated environmental toxicity. **Methodology:** This experiment lasted for a period of 28 days with 42 Wistar rats. The rats were randomly assigned into 7 groups of 6 rats. Group I received normal saline and water ad libitum. Group II were given lead acetate of 2mg/kg body weight. Group III and IV received 100mg/kg and 300mg/kg of aqueous extract and lead acetate respectively. Group V and VI received lead acetate and 100mg/kg and 300mg/kg of aqueous extract respectively. Group VII were given only the aqueous extract. All administrations were done orally using orogastric tube. **Result:** On examination of the organ, they showed inflammation, oedema, kupfer cells hyperplasia, mitochondria degeneration, Hydropic degeneration, necrosis, fatty changes, Glycogen reduction, etc. The above were observed to be reduced in the groups that received the extract especially the higher doses. The rats that received only the extract look healthier than all the other groups. **Conclusion:** *Ficus vogelii* may be of great importance in detoxifying the liver in the case of liver toxicity.

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INTRODUCTION

The environment is composed of various elements which contribute in making up the earth’s crust. These elements either affect the biological system positively or negatively depending on their nature (Pizzino, et al., 2014). One of the elements includes heavy metals which are vital part of the soil and while are beneficial others are deleterious to humans. About 40 of these heavy metals have been identified which are capable of combining with several organic molecules and are potential enzyme inhibitors because of their interaction with ligands present in proteins and can also deactivate enzyme system (Pizzino, et al., 2014). These metals include but not exclusively the following; Copper (Cu), Cadmium (Cd), Nickel (Ni), Zinc (Zn), Manganese (Mn), Arsenic (As), Lead (Pb), Iron (Fe), etc. As it is already a well known fact that some of these metals are very essential due to the biochemical and physiological functions they play in both plants and animal natural process (Pizzino, et al., 2014). According to Kumar and Agrawal, 2005; heavy metals have two major functions they are known for such as (i) they participate in redox reaction and (ii) they directly participate or are an integral part of several enzymatic reactions that either inhibit or enhance the biological processes. Some heavy metals like lead and cadmium have been reported to cause iron deficiency either by decreasing nutrient uptake or causing immobilization in roots (Rajesh and Madhoolika, 2005).

Lead has no physiological function to play in human body but is a very invasive material that affects several cellular processes and enzyme systems all over the body (Assi et al., 2016; Wani et al., 2015; ATSDR, 2007). Lead acetate induce toxicity is one of the leading factor which increases the generation of excessive reactive oxygen species (ROS) which is capable of inhibiting DNA repair, antioxidants production, enzyme reactions and damages nucleic acids (Assi et al., 2016; Patrick, 2006). It is a non biodegradable material in nature and so accumulates in the environment which leads to it constituting an increased hazard (Wani et al., 2015). The appearance of clinical manifestations from lead toxicity varies from individual to individual depending on the other several environmental factors. The Centre for Disease Control and prevention USA (CDC), 2012 reported a standard elevated blood level for adult as 10μg/dL and for children as 5μg/dL (CDC, 2012) instead of 10μg/dL that was previously suggested for children. Several reports including that of Advisory Committee for Children (ACC, 2012), have noticed that children are more susceptible to the effects of lead due to the fact that their organs are still in the developing stage and so have suggested that blood lead levels be set lower than what it is now and a frequent check must always be carried out especially where contamination is suspected. Lead can gain access into the body through several means such as ingestion of food water contamination, skin contact and inhalation. Once it enters the human body through any of the above means, it is quickly absorbed into the blood stream and its adverse effect is so vast that it affects almost all the systems of the body (Bergeson, 2008; Wani et al., 2015). In the blood stream lead has a first point of call which is the liver followed by the kidneys.

The liver is the largest internal organs of the body. The liver on its own performs several functions which include hematopoietic, glycogen storage drug detoxification, etc. In view of the above functions, anything that affects the histarchitecture of the liver may tend to impair those functions and expose the body to the danger of drug intoxication. This study was then designed to show the deleterious effects of heavy metal (lead) in particular in the liver histoarchitecture of adult Wistar rats and also access the roles of F.vogelii in restoring their integrity.

MATERIAL AND METHODS
Ethical clearance

The animals were subjected to the Guidelines of the Ethical Conduct in the Care
and Use of Animals, maintained at room temperature of 25±2°C with 12-hours dark-light cycle. These animals were fed with standard rodent diet and allowed water ad libitum (Alarifi et al., 2012; El-Nager and Aldahmash, 2013). The above guideline was strictly adhered to and thereafter we sort for and obtained the ethical clearance from the Faculty of Basic Medical Sciences University of Nigeria Enugu Campus.

**Experimental protocol**

Forty two healthy adult Wistar rats weighing between 180-200gm were used for this experiment and the animals were randomly assigned into 7 groups of six rats each after seven (7) days of acclimatization. Group 1 (untreated group) that received normal saline and water ad libitum. Group 2 (lead acetate control) that were given lead acetate in a dose of 2.5mg/kg body weight for 28 days. Group 3 (protective low dose) that received 100mg/kg dose of aqueous extract and lead acetate by the same route for 28 days. Group 4 (protective high dose) that received 300mg/kg dose of aqueous extract and lead acetate orally for 28 days. Group 5 (currative low dose) that received lead acetate by oral route and 100mg/kg dose of aqueous extract and lead acetate orally for 28 days. Group 6 (currative high dose) that were given lead acetate by the same route and 300mg/kg dose of aqueous extract for 28 days. Group 7 (Aqueous extract only) that were given lead acetate by the same route and dose for 28 days. This dose was prepared by dissolving 2.5mg of lead acetate in1000ml of distilled water (Jin, et al., 2008). All the animals were administered to using orogastric tube.

**Collection and extraction leaves**

Fresh leave of *F. vogelii* was collected from Enyibichiri Ndufu-Alike Ikwo in Ikwo Local Government Area of Ebonyi State. The leaves were washed and dried in ventilated room. Thereafter, it was crushed into powder using electronic blender and passed through mesh sieve to get the fine powders. The powder was divided into A and B, A was used for aqueous extraction while B was used for ethanolic extraction (Sasidharan et al., 2011). Five hundred grams (500g) of the powder was weighed using an electronic weighing balance and soaked in 1200mL of water (powder/solvent). The mixture was agitated using an electric blender to enhance proper mixing of the solvent with the powder and then poured into air-tight plastic containers. The container with the mixture was kept in a refrigerator for 48hours. The mixtures were filtered first with cheese cloth and then with Whiteman No 1 filter paper (24cm). The filtrates were separated and concentrated in vacuum using Rotary Evaporator to 10% of their original volumes at 37°C - 40°C. These were concentrated using a water bath until a sticky paste was gotten. The extracts were stored in a refrigerator at a very low temperature until it is required for use. All preparations were performed at the Department of Anatomy Faculty of Basic Medical Sciences, College of Medicine University of Nigeria Enugu Campus (UNEC).

**Histological Study**

At the end of the experiment, the rats were starved overnight and anaesthetized and then decapitated (Schoenwolf et al., 2015). The animals were dissected and the liver was removed and quickly fixed in 10 % formalin for routine histological procedures. The tissues were processed and embedded in paraffin wax. Thin sections of 4-5μm were obtained and stained using haematoxylin and eosin (H&E) and were examined under light microscope to determine the histological changes in the liver histology.

**RESULTS**

In this experiment, all the rats were closely and carefully monitored and the administration was done orally. We did not record any case of mortality in all the groups during the period of study. There animals were observed to feed well without any rejection of their diet in all the groups both control and experimental.
Photomicrographic plates

1a

1b

2a

2b

3a

3b

4a

4b
Fig. 1. Normal structure of liver showed normal central vein (CV), normal kupfer cells, normal hepatocytes and portal triad (PT). H and E stains, 1a- x100 and 1b- x400. Fig. 2: Photomicrograph of rats that received only lead acetate shows sinusoidal hemorrhage, infiltration, dilatation of central veins and vacuolar degeneration of hepatocytes and congested central vein. H and E stains, 2a- x100 and 2b- x400. Fig. 3: Low dose of the curative extract showing dead kupfer cells, central vein oedema, inflammations, congested central vein, dilated central vein and swollen nuclei of hepatocytes. H and E stain 3a- x100 and 3b- x400. Fig. 4: High dose of the curative extract showing little stains of blood, near normal sinusoidal cells and cell regeneration. H and E stain 4a- x100 and 4b- x400. Fig. 5: Low dose of the protective extract showed healthy central vein, binucleated cells and small amounts of collagen fiber, congested central vein (CV), nuclear degeneration, dilated blood sinusoids, lymphocyte infiltration, collagenous fibers and degenerated cells. H and E stain 5a- x100 and 5b- x400. Figure 6: High dose of the protective extract healthy hepatocytes, necrotic foci (red arrow), kuffer cells. H and E stain 6a- x100 and 6b- x400. Figure 7: The liver of rats that received only the aqueous extract showed normal kupfer cells, clear central veins and the liver sinusoids are normal. H and E stain 7a- x100 and 7b- x400.
Histological study of the kidney

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7
Fig.1. Normal structure of kidney showed normal glomerulus, calyx and normal medullar; H and E stain, x400. Fig.2: Photomicrograph of rats in the positive control showing necrotic glomerulus, Optical empty spaces, oedema, hemorrhage, inflammations; H and E stain x400. Fig.3: Low dose of the curative extract showing cell regeneration, clearing oedema and areas of healing x400. Fig.4: High dose of the curative extract showing little stains of blood, near normal glomerulus and cell regeneration; H and E stain, x400. Fig.5: Low dose of the protective extract showed healthy glomerulus, binucleated cells and small amounts of collagen fiber, cell degeneration; H and E stain, x400. Fig. 6: High dose of the protective extract showed healthy glomerulus, normal cells, regeneration of cells, cleared oedema; H and E stain, x400. Fig. 7: This received only aqueous extract and showed normal kidney cells, glomerulus, cleared oedema and looks more vascularized; H and E stain, x400.

DISCUSSION

Histology of the liver

One of the first organs to be exposed to anything that enters the body as nutrients or other xenobiotics is the liver through the portal vein (Al- Ubaidy et al., 2006; El Sayed et al., 2014). According to Al-Ubaidy et al., 2006, the liver is made up of highly active metabolic tissue consisting of huge complement of detoxification machinery system. When a very high dose of lead acetate is administered to animals, it results in highest lead accumulation in the following organs kidney then liver, bone marrow, brain and finally in the heart muscles (El Sayed et al., 2014). This is why lead is the most invasive heavy metal known to man. Frangenberg, 1986 stated that “Chronic oral administration of low doses of lead results in its accumulation particularly in bone marrow, kidney and skeletal muscles.” The liver being one of the major organs involved in the storage, biotransformation and detoxification of toxic substances, is of relevance in heavy metal poisoning (Herman and Geraldine, 2009). Absorbed lead is stored in soft tissues mainly in the liver (Patrick, 2006) via the portal vein. With the above the live serves as the best organ that can be used to examine the structural changes that reflect possible toxic lead effects Abdou and Newairy, 2006; Suradkar et al., 2010).

In this present research, we decided to look at the effect of the same dose of lead (2.5mg/kg) on seven groups of adult Wistar rats and the role of Ficus vogelii leave extract in detoxifying the liver. Our results in this research are in agreement with other studies showing that exposure of rats to lead acetate causes a decrease in body weight (Annabi et al., 2007; Ibrahim et al., 2012; Shakoor et al., 2000; Xia et al., 2010). The decrease in body weight may not only be a result of reduced food consumption, but also from direct toxicity of the lead acetate, perhaps by malabsorption of nutrients from toxic effects on the gastrointestinal tract or by inhibition of protein synthesis according to Hammond et al., 1990; Minnema and Hammond, 1994. Examination of the liver tissues using light microscope revealed various alterations such as Portal triads inflammation, Kupfer cells hyperplasia, Nuclear vesiculation, Nuclear pyknosis, binucleation, Cytoplasmic inclusions, Necrosis, Hydropic degeneration, Fatty changes, hemosiderosis, Glycogen reduction, medullary hematopoiesis and Alkaline phosphatase activity. These alterations induced by lead as seen in the nuclei of the hepatocytes might be due to increased cellular activity and nuclear interruption in the mechanism of lead detoxification.

The lead intoxication induced various alternations in the liver cells nuclei and cytoplasm as seen in the photomicrographs of the present study. This might be attributed to accentuated activities of the hepatocyte and nuclear interruption in the mechanism of lead detoxification. Gerlyng et al., 2008; Bhargava and Schnellmann, 2017 described a similar bi-nucleation seen in the liver of the experimental rats which may be due to cell injury and a sort of chromosome hyperplasia. This is often seen regenerating cells. There were also appearance of inflammatory cells in the hepatic tissues that
received only lead which might be as a result of lead intoxication. This agrees with the report of Johar et al., 2004; Bhargava and Schnellmann, 2017, when they stated that lead could interact with enzyme and protein of liver tissues to interfere with defense mechanism to generating reactive oxygen species (ROS) which initiates inflammatory response. The necrosis, hemorrhage and vacuolation seen in this study due to intoxication as presented in the photomicrographs has been reported by several authors such as Bashir and Noory, 2012; Pandey et al., 2008; Abd El-aal et al., 1989.

It was observed that administration of the extract reduced the effects of the intoxication caused by lead in the liver of rats. As shown in figure 4, this may be a proved that the extract as an antioxidant is good for consumption. There were still some necrotized areas observed in the tissues as seen in figure 3 but not as conspicuous as those in figure 2 that received only lead. The tissues appear more vascularized which may be due to the ability of the extract to restore the lost blood supply to the points whose supply were lost due to toxicity (Patra et al., 2001). The hemorrhage that appeared to be scattered in the groups treated with only lead acetate was reduced n other groups represented by figure 5 and 6. Figure 7 above reveals the effective nature of the extract as it represents the group that received only the extract without hemorrhage, necrosis, congested central vein and normal hepatic cells.

Histological examination of the Kidney

The kidney is one of the most vital organs required by the body to perform its several important functions such as maintenance of homeostasis, excretion of toxic metabolites, assists in detoxification and regulation of extracellular environment (Ferguson et al., 2008). Nephrotoxicity has become a major health challenge in our present age and according to Kim and Moon, 2012; 20% of it are induced by some chemicals ingested into the body such as drugs, but in the elderly, the prevalence is increased up to 66% by medication as life span increases.

In this experiment various pathological changes were observed in the kidney such as thickened and congested blood vessels, intertubular hemorrhages, intertubular infiltration, congested and atrophied glomeruli, hyper chromatic nuclei, hyperplasia of tubular epithelium, desquamated tubular epithelial cells.

In a healthy young adult, the glomerular filtration rate (GFR) is 120 ml per minute and can keep a constant filtration rate as well as maintain the displacement of urine through regulation of blood flow in afferent and efferent arteries for adjustments or maintenance of intraglomerular pressure (Kim and Moon, 2012, Bulacio et al., 2015). Anything that affects the glomerulus will directly endanger the general functionality of the kidney. Based on the damage caused to the glomerulus in this research, lead is capable of inducing nephrotoxicity. This was reduced by the administration of the extract. The renal tubules, especially proximal tubule cells, are exposed to chemicals as the kidney concentrates and reabsorbs substances and they are greatly affected by this toxicity (Kaminski et al., 2017; Perazella, 2005). This will cause damage to the mitochondria in tubules and destabilize tubular transport system and at the same time increase the oxidative stress by generating more free radicals. This is in agreement with the work carried out by Zager, 1997; Markowitz and Perazella, 2005. One other major problem that is very common in nephrotoxicity induced by chemicals (lead) is inflammation of the glomerulus, proximal tubules and surrounding kidney cellular matrix. According to Karpman et al., 2017; Kim and Moon, 2012, inflammation that disturbs normal kidney functions and induces toxicity includes glomerulonephritis, acute and chronic interstitial nephritis and this were seen in the tissues as they were edemas scattered all over.

CONCLUSION

The result of this study might be suggesting that Ficus vogelii leave can help in the detoxification of the detoxifiers (kidney and liver). The leave may be used to counter the
effects of lead toxicity and could be beneficial in helping to rebuild the worn out cells or tissues. Hence, a daily portion in the meal is recommended for individuals at risk of lead intoxication.

REFERENCES
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