

Evaluation of the Effect of Sub Chronic Lead Poisoning on Bodyweight and Weight of Vital Metabolic Organs in Adult Wistar Rats; and the Role of *Launaea Taraxacifolia*

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Abstract

Background and Aim: Lead is a noxious and accumulative substance in the body system when it exceeds certain threshold concentrations. Its toxicity is multi-systemic. It is associated with a number of physiological, biochemical, and morphological changes, however, discrepancy in organ weight is usually affected by relative weight gain. This study revealed the relationship between sub chronic lead intoxication, vital metabolic organs and the role of treatment on organ changes.

Methodology: Seventy three Albino rats of 10 to 12 weeks old weighing 180- 200 grams were used for the experiment. The experimental albino rats were divided into 10 groups (n = 6). One group was allowed free access to distilled water only, while eight groups were allowed free access to lead acetate (2 mg/ml) in drinking water, for 5 weeks consecutively. Seven out of the eight groups were later administered *Launaea taraxacifolia* (LT) extract (50, 100 and 200 mg/kg, p. o), DMSA (Dimecarptosuccinic acid), DMSA + LT, DMSA + VC and VC only for 21 days consecutively. The tenth group was administered LT (100 mg/kg) for 21 days, and later allowed free access to lead acetate in drinking water (2mg/ml) for 5 weeks. The animals were treated and monitored for 35 days and their body weight was taken weekly. Animals were sacrificed on the next day after completion of the duration. Blood was collected for BLL analysis, while respective body organs (liver and kidneys) were taken out for relative organ weight; liver was excised, cleared of adhering fat and weighed. The organs were later subjected to histological studies.

Results and Discussion: There was reduction in weight of group treated with Pb and Pb + LT. but there was reduction in weight of the liver and kidneys in group treated with Pb only. However, the study revealed pathological changes and reversal of histological changes in groups treated with DMSA, LT and combination therapy (DMSA+LT, DMSA+VC); which was likely to be due to the effect of antioxidant on sub chronic lead poisoning.

Conclusion: The study revealed discrepancies in organ weight with body weight but not significant; however, there was reversal of pathological changes with three doses of *Launaea Taraxacifolia*.

Keywords: Lead Poisoning; Body Weight; Organ Weight; *Launaea Taraxacifolia*; Dimecaptosuccinic Acid

Introduction

Lead is a well - known toxic environmental contaminant, resulting in mild to life threatening health problems [1]. There is no amount of lead that is safe in blood; therefore, as lead exposure increases, severity of symptoms and effects also increases. However, body changes over time depends on its threshold and concentration [2]. Hazardous effect of lead result known as Lead poisoning is identified by WHO is a major global health concern that warrants urgent intervention through environmental management of heavy metals; thus, reduction of their release to air, water and soil will ultimately minimize their adverse impacts on human health and the environment [3,4].

Lead has major effects on different parts of the body, as a toxicant, it can accumulate in the blood and cause metabolic disturbances and/or organ intoxication. The accumulation of these substances may enhance morbidity and mortality of affected patients. Its multi-systemic health effects are usually associated to the duration and extent of exposure. It could be acute or chronic. Acute exposure is usually fatal in children because lead crosses due to immature blood brain barrier [5,6]. Liver and kidneys are the major metabolic organs of lead and are frequently affected in subacute exposure. However, chronic exposure of lead may result in mental retardation, birth defects, psychosis, autism, allergies, dyslexia, weight loss, hyperactivity, paralysis, muscular weakness, neurological damage, renal damage and may even cause death [7,8].

Although, there was no significant body change during acute exposure but the effect of sub chronic lead poisoning on body weight and organ weight cannot be underestimated. However, the comparison of the organ weights of treated animals with untreated animals is often complicated by differences in body weights between

groups. Therefore, different parameters are commonly used for analysis of organ weight are the ratio of the organ weight to body weight (to account for differences in body weight) and the ratio of the organ weight to the liver/kidney weight [9,10].

Meanwhile, organ changes are suggested to be due to significant alteration in oxidative stress-related indices, appreciable cytotoxic changes leading to altered organ cells responsiveness to apoptosis. Among vital organs, liver was the most affected in case of weight changes [11,12]. Heavy metal toxicity has been documented to increase liver weight by induction of ROS resulting in DNA strand breaks and lipid peroxidation [13]. Meanwhile, it has been reported that plants develop a complex mechanism by which they control the uptake and accumulation of heavy metals [14]. Leaf of *Launaea taraxacifolia* is of high nutritional and medicinal value [15]. Studies have revealed the ameliorative and protective effect of *L. taraxacifolia* leaves on lead poisoning [16,17]. Also, studies on the nutritional value and phytochemical characterization of *L. taraxacifolia* revealed that extracts of its leaves were rich in micro nutrients and antioxidants that are likely responsible for ameliorative and protective effect of oxidative stress caused by lead toxicity [18,19].

Materials and Methods

Lead acetate (ACS reagents, grade > 99 % pure) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Lead toxicity was induced through ingestion of lead acetate in drinking water for 5 weeks consecutively. Lead acetate as a powder was dissolved in distilled water 2 mg/ml daily [11]. Animals drank from the graduated water bottle and the decrement in the amount of water over 24-h was measured by subtracting the day's reading from the amount from previous. *Launaea taraxacifolia* was collected at Oranfe area of Ile-Ife, Osun State, Nigeria. It was

identified and authenticated by a taxonomist at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State. It was extracted with methanol, to obtain methanol extract. The yield was 28.27 g which is 9.42 %w/w of the powdered material. The powdered form was subjected to cold extraction.

Seventy three Albino rats were used for the experimentation. Rats of 6 to 10 weeks old weighing approx. 180- 200 grams were breed in animal house, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State. Animals were kept under conventional condition in a metabolic cage (12 hr. light to dark cycle) and were made available to standard rat food and tap water ad libitum. The experimental albino rats were divided into 10 groups (n=6). One group was allowed free access to distilled water only, while eight groups were allowed free access to lead acetate (2 mg/ml) in drinking water, for 5 weeks consecutively. Seven out of the eight groups were later administered *Launaea taraxacifolia* (LT) extract (50, 100 and 200 mg/kg, p. o), DMSA, DMSA + LT, DMSA + VC and VC only for 21 days consecutively. The tenth group was administered LT (100 mg/kg) for 21 days, and later allowed free access to lead acetate in drinking water (2mg/ml) for 5 weeks. The animals were treated and monitored for 35 days and their body weight was taken weekly. Animals were sacrificed on the next day after completion of the duration. Blood was collected for BLL analysis, while respective body organs (liver and kidneys) were taken out for relative organ weight. Liver was excised, cleared of adhering fat and weighed. The organs were later subjected to histological studies.

Histopathological Studies

Liver and the two kidneys were carefully fixed in 10% formal saline solution to prevent putrefaction and autolysis. They were counter stained with eosin and viewed under the microscope with photomicrographs taken with a Leica DM750 Camera Microscope (X 400). The organs were examined microscopically by pathologists, and were subjected to review by an anatomist.

Statistical Analysis: The results obtained were presented as mean \pm SEM (Standard error of the mean). Comparisons were made between treated and control groups using Student's *t*-test. One-way ANOVA was used to analyse significant difference of means with Student Newman Keuls post hoc test for multiple comparisons. Results obtained were presented as mean \pm SEM (standard error of the mean), level of significance was taken as $p < 0.05$.

Results and Discussion

Eating and drinking are normal characteristics of living things. The two important activities are triggered by hunger, thirst and satiety feeling. In this study, water and food intake was observed in all the groups. Clinical observations showed that animals treated with lead only were aggressive and irritable throughout the period of the experiment. There was mortality in group treated with lead only, two animals died. The mean volume of average water intake during the first five weeks in the group administered ascorbic acid was significantly increased (37.79 ± 1.25 ml), $p < 0.05$ when compared with lead only rat. While values of all the other treated groups were in significant when compared with Pb only group. Likewise, there was no significant change in *p* value of Pb when compared with VEH (Distilled water only), $p < 0.05$.

The average water intake during the first five weeks of the experiment, ranged between 30 – 40 ml/day. The average food consumption ranged between 20 -30 g/day. There was significant ($p < 0.05$) values of feed consumption in group treated with DMSA + LT when compared with Pb treated group only, $p < 0.05$. However, there is no significant change in all the other groups when compared with Pb only group. Similarly, there was no significant change in feed consumption in group administered distilled water when compared with the group treated with Pb only.

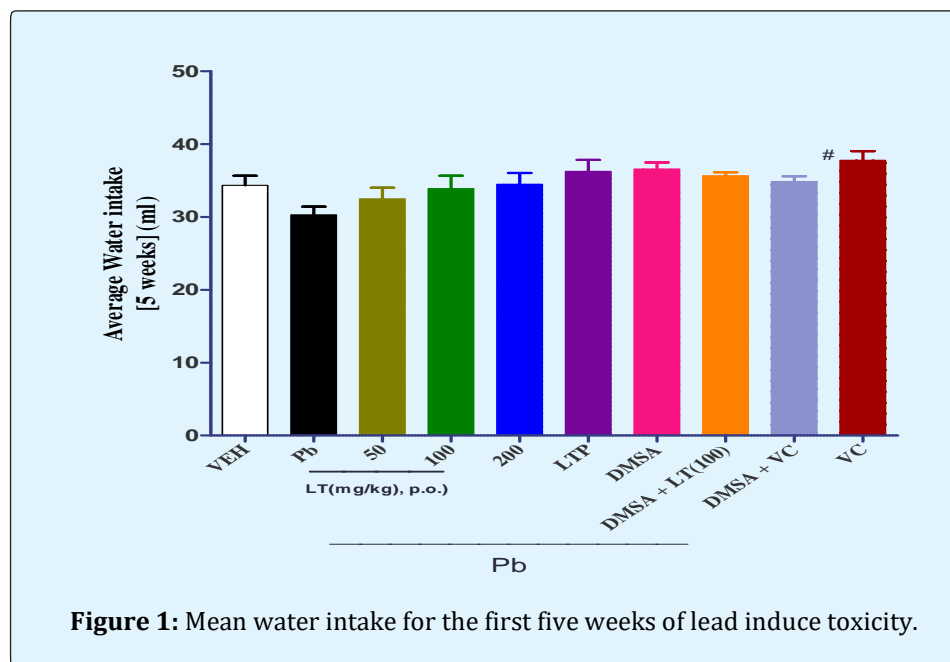
Although there was no significant changes in body weight, change in weight was observed in all the groups; there was reduction in the body weight in those treated with Pb only, DMSA, extract of 50mg/kg and combination therapy while there was increase in body weight of those administered distilled water only, 100mg/kg and 200 mg/kg of LT. Likewise, there was no significant change in the ratio between body weight, weight of right and the left kidney. However, there was significant decrease ($P < 0.05$) in ratio of body weight and the liver in group treated with Pb only. After 21 days of treatment with the DMSA, combination therapy and three doses of LT, there was reversal of liver histoarchitecture and minimal renal distortion in fig 3 and 4.

This study showed that, continuous administration of Pb without treatment contributed to decreased weight compared with other group control group ($P < 0.05$). Lead has hepatotoxic effect resulting in liver cell damage resulting to increase in serum levels of AST and ALT [20,21].

This present study showed that Pb is a major stress inducing agent on hepatocytes. Moreover, these results concur with previous studies that reported an elevation in AST and ALT levels after treatment with lead caused by acute hepatitis, jaundice, and liver cirrhosis [22,23]. Kidney is a major metabolic and excretory organ; lead accumulation was excreted via the urine. However, proximal convoluted tubule of kidney may become a target for the toxic substance to which the organism is exposed [24-26]. Oxidative stress has been implicated to play a role, at least in part, in pathogenesis of many disease conditions and toxicities in animals. Lead oxidative stress is a reflection in imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage as a result of overproduction of reactive oxygen species and free radicals beyond the cells intrinsic or functional capacity to neutralize toxic substance in the body, thus leading to DNA fragmentation, protein and lipids oxidation [27-29]. However, methanolic extract of *L. taraxacifolia* leaves has been reported to have a protective feature for DNA, lipid and protein due to its antioxidant activity [30,31].

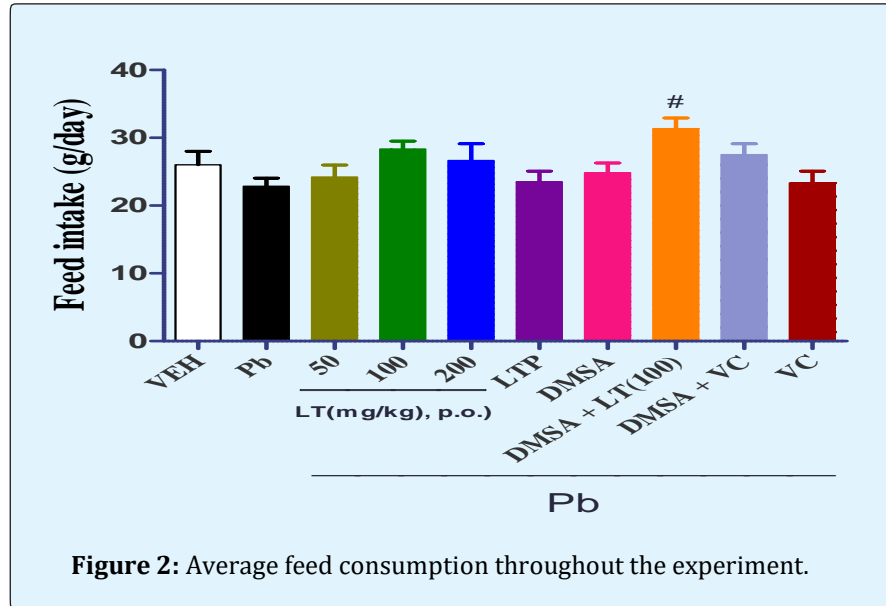
Meanwhile, the present study reversal of oxidative stress by the extract was also demonstrated, the effect is seen on histopathological view of hepatic and renal tissues. LT improves histopathological changes in both liver and kidney caused by lead intoxication. Pre-treatment with *L. taraxacifolia* extract at a dose of 100 mg/kg body weight for 21 consecutive days protect rats against the lead acetate induced injury. According to Eluwole, et al. histological examination of liver tissue in rats groups treated with daily dose of 100 mg/kg of LT for 21 days revealed that, most of the histological alterations induced in lead acetate treated groups were markedly reversed by *Launaea taraxacifolia*. The photomicrographs of all the treated groups showed minimal alterations when compared to the lead acetate treated rats.

The below figure shows the mean value of lead in drinking water for 5 weeks. VEH = Distilled water only, Pb = Lead, DMSA = Dimecarptossuccinic acid, VC = Vitamin C or Ascorbic acid. LT = *Launaea taraxacifolia*, LTP = 100 mg/kg of *Launaea taraxacifolia* before lead. # means that p value was significant when compared with Pb group only, $p < 0.05$.



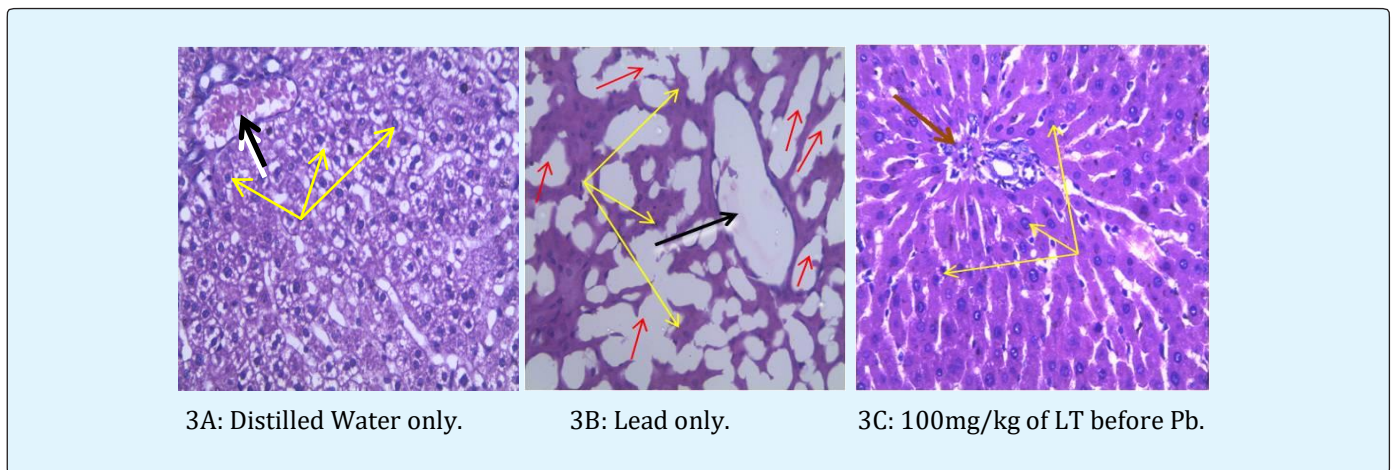
The below figure shows the average feed consumption throughout the experiment. VEH = Distilled water only, Pb = Lead, DMSA = Dimecarptossuccinic acid, VC = Vitamin C

or Ascorbic acid. LT = *Launaea taraxacifolia*, LTP = *Launaea taraxacifolia* before lead. # means that there was significance when compared with Pb group only, $p < 0.05$.



Figures 3A - 3F: Figure **A** possesses radially arranged hepatocytes (yellow arrows) around the central vein (black arrow) but **B** shows various degrees of vacuolation (red arrow). Microscopically, coat of the central vein of figure **C** and **E** were thickened (brown arrow) and reduced central vein (brown arrow). However, **F** has disorganized outline with very few hepatocytes, but normal central vein (black arrow). All the figures apart from **A** and **E** possess vacuolated cytoplasm. Pb - Lead, LT- *Launaea taraxacifolia*, LTP- 100 mg/kg of *Launaea taraxacifolia* before lead.

Figure **3G** and **3H** have normal microstructure, i.e., normal hepatocytes (yellow arrows) and central vein (black arrow), figure **H** shows various degrees of vacuolation (red arrow), while thickening of venous wall and necrosis was seen in figure **J** (white arrow), coat of the central vein of figure **K** was thickened (green arrow). However, figure **L** possesses well spread out hepatocytes which are radially arranged around the central vein. Pb- Lead, LT - *Launaea taraxacifolia*, DMSA - Dimercaptosuccinic acid, VC - Vitamin C.



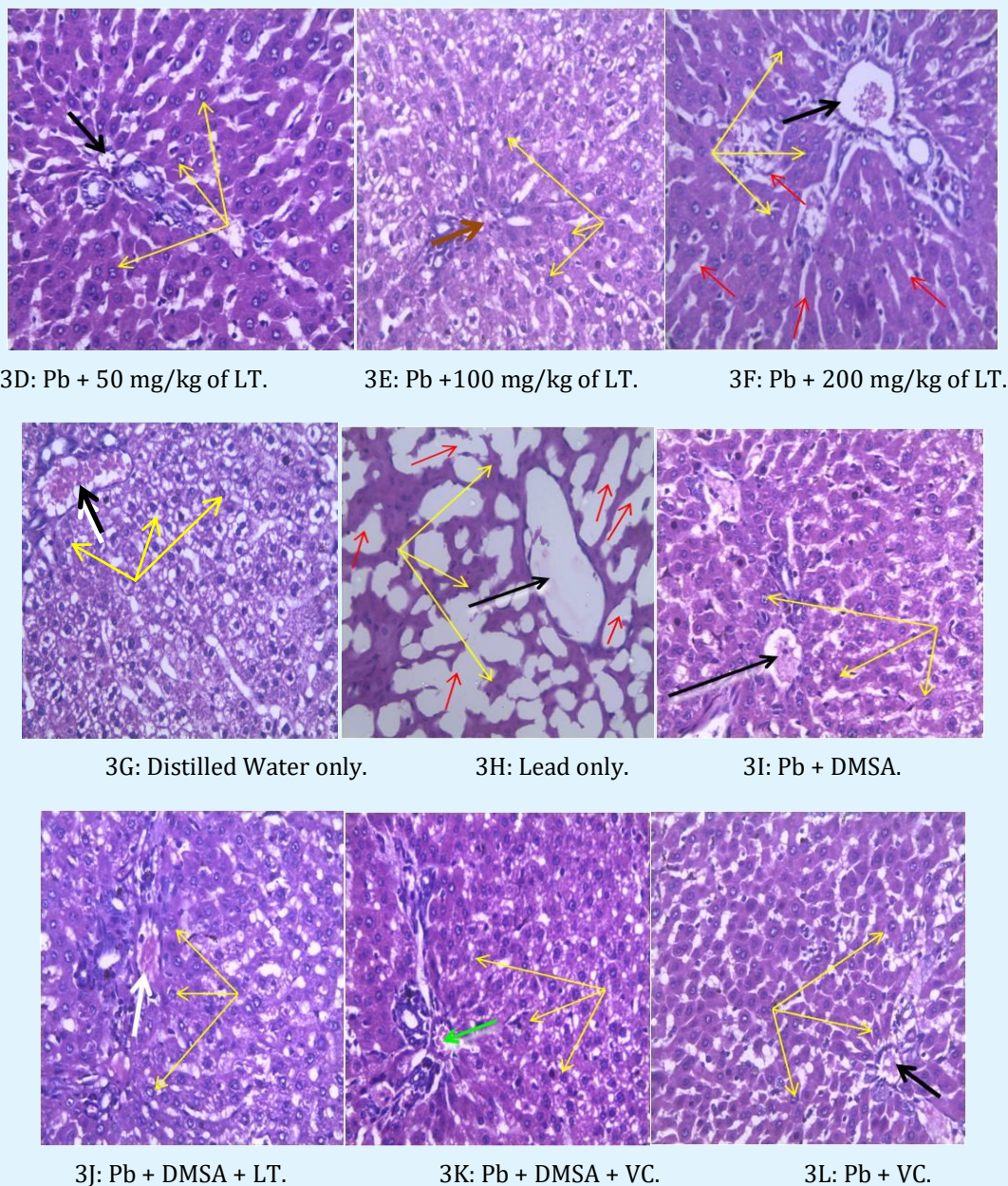
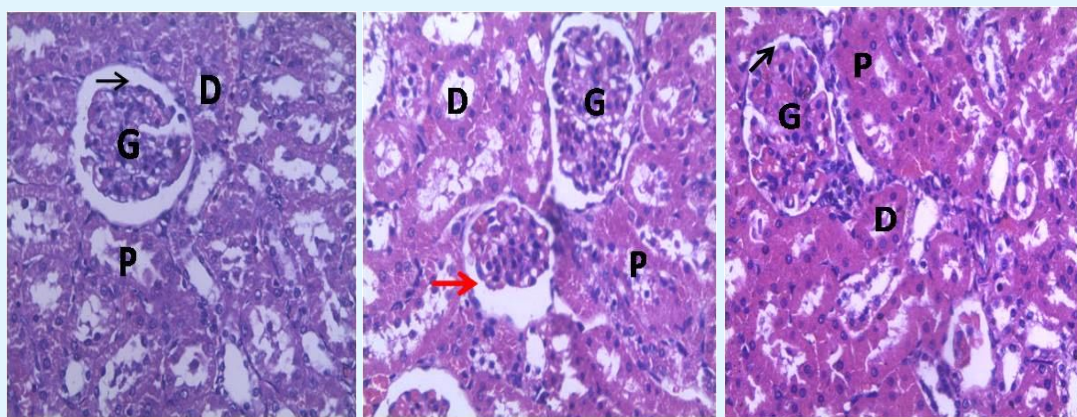


Figure 3: Photomicrograph of the Liver. X 400 (Contd).

Figure 4A- 4F: All the slides show normal histo-architecture, no distortion. However, there was evidence of glomerular shrinkage (red arrow) shown by increase in Bowman's space in Fig. 4B, while the other groups had

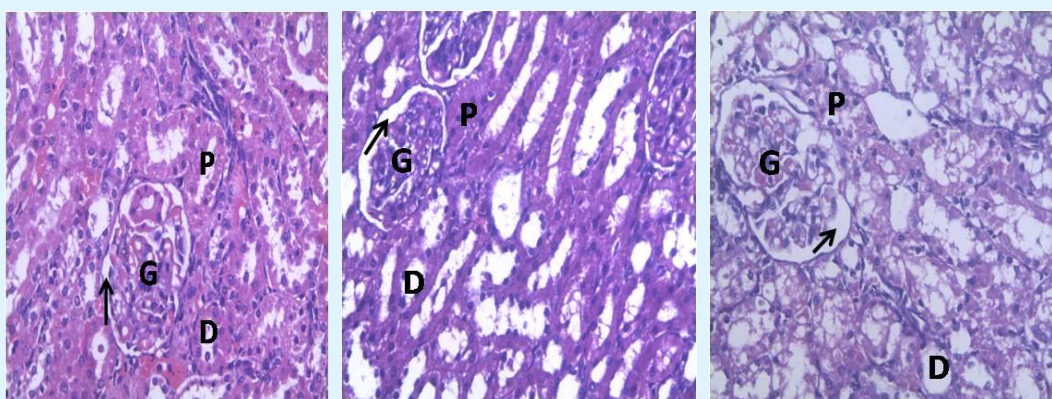
intact Bowman's capsular space (black arrow), glomeruli (G) with normal appearing distal (D) and proximal (P) convoluted tubules. Pb - Lead, LT - *Launaea taraxacifolia*.



4A: Distilled Water only.

4B: Lead only y.

4C: 100mg/kg of LT before Pb.



4D: Pb + 50 mg/kg of LT.

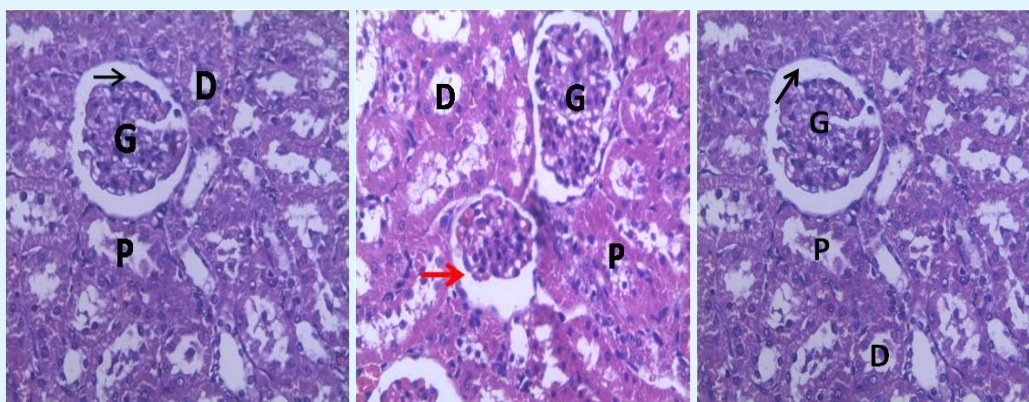
4E: Pb + 100 mg/kg of LT.

4F: Pb + 200 mg/kg of LT.

Figure 4: Photomicrograph of the kidney x 400.

Figure 4G-4L: Apart from 4H, all the slides possess similar characteristic with no distortion of renal parenchyma, glomeruli (G) were intact with normal Bowman's capsule

(Black arrows). Pb - Lead, LT- *Launaea taraxacifolia*, DMSA - Dimecaptosuccinic acid, VC -Vitamin C or ascorbic acid.



4G: Distilled Water only

4H: Lead only

4I: Pb + DMSA

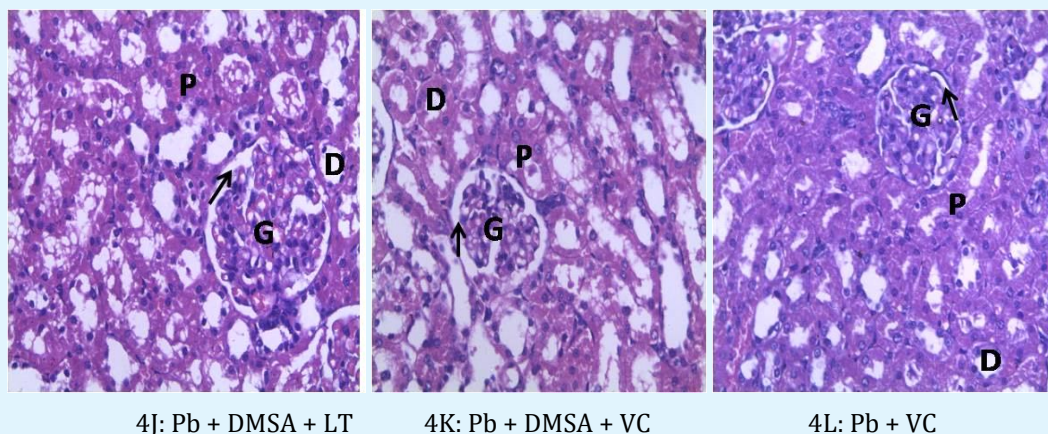


Figure 4: Photomicrograph of the kidney X 400 (Contd.)

VEH = Distilled water only,
 Pb = Lead,
 DMSA = Dimecarptosuccinic acid
 VC = Vitamin C
 LT = *Launaea taraxacifolia*
 LTP = 100 mg/kg of *Launaea taraxacifolia* before lead treatment

Groups	Initial Body Weight (G)	Final Body Weight (G)	Liver Weight (G)	Right Kidney Weight (G)	Left Kidney Weight (G)
VEH (No Lead)	182 ± 5.20	191.80 ± 3.40	5.66 ± 0.05	0.66 ± 0.05	0.48 ± 0.03
Pb only.	191 ± 2.57	189.50 ± 2.49	3.41 ± 0.04	0.41 ± 0.04	0.41 ± 0.04
Pb + LT*	181 ± 2.31	187.40 ± 3.0	6.73 ± 0.34	0.67 ± 0.04	0.65 ± 0.03
Pb + 50 mg/kg LT	183 ± 4.24	176.20 ± 5.79	5.82 ± 0.43	0.57 ± 0.06	0.57 ± 0.06
Pb + 100 mg/kg LT	189 ± 1.48	191.10 ± 2.98	6.11 ± 0.34	0.61 ± 0.07	0.59 ± 0.06
Pb + 200 mg/kg LT	183 ± 4.01	187.10 ± 5.20	6.54 ± 0.06	0.54 ± 0.06	0.61 ± 0.05
Pb+DMSA only	184 ± 3.70	180.70 ± 4.71	5.57 ± 0.38	0.57 ± 0.04	0.61 ± 0.07
Pb+ DMSA +VIT. C	184 ± 2.46	181.90 ± 3.30	5.41 ± 0.23	0.66 ± 0.05	0.70 ± 0.04
Pb+DMSA+ LT	185 ± 2.50	182.60 ± 4.67	4.70 ± 0.04	0.70 ± 0.03	0.57 ± 0.07
Pb + VIT. C Only	180 ± 4.24	182.0 ± 6.34	5.73 ± 0.22	0.48 ± 0.03	0.50 ± 0.03

Table 1: Effects of *L. taraxacifolia* body weight and organ weight (liver and kidneys) in sub chronic lead poisoning.

Conclusion

This study shows that, sub chronic phase of lead intoxication may not significantly affect the body weight and weight of the vital organs (kidneys and liver), but silently affects the histological structure of both liver and kidney. However, the histological alterations were reversed by *Launaea Taraxacifolia*.

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Ethical Approval

This study was approved by the University Research Committee (URC) on 25th of October, 2016 with registration number, PHP/12/13/H 0602.

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