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Lead-Induced Injury, The Antioxidant Role of Thaumatococcus Danielli in Adult Wistar Rats.

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Abstract: *Thaumatococcus danielli (Benn). Benth, a member of the Amaranthaceae family has continued to be of immense benefit to the people in the tropics, especially in Nigeria. The leaves are widely used among the Yorubas as a wrapping leaf and for the management of diabetic Mellitus. It is an economic plant with versatile uses in southern Nigeria. The arils attached to the seed contain thaumatin, a non-sugar sweetener, and a taste modifier. Thaumatococcus Danielli is a plant species from Africa, known for being the natural source of thaumatin, an intensely sweet protein that is of interest in the development of sweeteners. This study is aimed at evaluating the haematological and histological effect of thaumatococcus danielli on lead-induced toxicity in the liver and kidney of Wistar rats. Thirty rats(30) were used for this study, divided into 5 groups with 6 in each group. Group A was the control, group B was given lead only, groups C and D were given lead and Thaumatococcus danielli, and group E was given Thaumatococcus danielli only. This study revealed that oral ingestion of lead for the period of 21 days lead to inflammation, necrosis, and hepatotoxicity of the liver and kidney of the laboratory animals both histologically and biochemically and the treatment of lead-induced toxicity with Thaumatococcus daniellii methanolic leaf extract has a significant effect on the hematological parameters at the dose administered.*

Keywords: *Liver,Lead,Thaumatcoccus danelli,kidney,hepatotoxicity,*

1. INTRODUCTION

Lead (Pb) is ubiquitous and one of the earliest metals discovered by the human race. Unique properties of lead, like softness, high malleability (ability to bent, form, or shape without cracking or breaking), ductility, low melting point, and corrosion resistance, have resulted in its widespread usage in different industries like automobiles, paint, ceramics, plastics, etc. Lead was used in making water pipes in the Roman Empire[1], its ease of working and corrosion resistance ensured its widespread use in other applications, including pharmaceuticals, roofing, currency, and warfare[2].

This in turn has led to a manifold rise in the occurrence of free lead in a biological system and the inert environment[3]. Lead is regarded as a potential occupational toxin and its toxicological manifestations are well known. The non-biodegradable nature of lead is the prime reason for its persistence in the environment. Human exposure to lead occurs through various sources like leaded gasoline, industrial processes such as lead smelting and coal combination, lead-based solder in water supply systems, battery recycling, grids, and bearings, etc. Although lead toxicity is a highly explored and comprehensively published topic, complete control and prevention over lead exposure are still far from being achieved. There is no such level of lead that appears to be necessary or beneficial to the body and no 'safe' level of exposure to lead has been found[3].

Lead Toxicity is a particularly insidious hazard with the potential of causing irreversible health effects. It is known to interfere with several body functions and it is primarily affecting the central Nervous, Haematopoietic, hepatic, and renal systems producing serious disorders [4]. Acute toxicity is related to occupational exposure and is quite uncommon. Chronic toxicity on the other hand is much more common and occurs at blood lead levels of about 40-60 µg/dl. It can be much more severe if not treated

in time and is characterized by persistent vomiting, encephalopathy, lethargy, delirium, convulsions, and coma [3][5].

Lead directly affects the hematopoietic system by restraining the synthesis of hemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway. It also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes. The combined aftermath of these two processes leads to anemia[6][7]. Anemia caused on account of lead poisoning can be of two types; hemolytic anemia, which is associated with acute high-level lead exposure, and frank anemia, which is caused only when the blood level is significantly elevated for a prolonged period[8].

Lead significantly affects the heme synthesis pathway in a dose-dependent manner by downregulating three key enzymes involved in the synthesis of heme. δ – aminolevulinic acid dehydratase (ALAD), a cytosolic enzyme that catalyzes the formation of porphobilinogen from δ-aminolevulinic acid (ALA), Amino Levulinic Acid Synthetase (ALAS), a mitochondrial enzyme that catalyzes the formation of aminolevulinic acid (ALA), and finally the mitochondrial enzyme ferrochelatase that catalyzes the insertion of iron into protoporphyrin to form heme[9]. The initial and final steps of heme synthesis take place in the mitochondria, whereas the intermediate steps take place in the cytoplasm. Lead inhibits the three aforementioned vital enzymes of this pathway but its effect on ALAD is more profound and its inhibition has been used clinically to gauge the degree of lead poisoning. Inhibition of ALAD results in the accumulation of aminolevulinic acid, detectable in the plasma and urine even at blood levels less than 10µg/dl. Heme biosynthesis does not decrease until the activity of ALAD is inhibited by 80-90%, which occurs at a much higher blood lead concentration of about 55 µg/dl [10]. Inhibition of ferrochelatase result in increased excretion of coproporphyrin in urine and accumulation of protoporphyrin in erythrocytes, moreover, inhibition of this enzyme results in the substitution of iron by zinc in the porphyrin ring forming zinc protoporphyrin (ZPP). The concentration of ZPP thus gets increased, which can also be used as an indicator to monitor the level of lead exposure [11]. Thus, the collective inhibition of these three key enzymes blocks heme production via the heme synthesis pathway. The mechanism responsible for shortening the life cycle of erythrocytes is not well understood. One of the earliest observed hematological effects of lead revealed basophilic stippling of red blood cells (presence of dense material in red blood cells), which is also a potential biomarker for the detection of lead poisoning. These aggregates are degradation products of ribonucleic acid[12].

Thaumatococcus danielli (moi moi leaf) *Thaumatococcus danielli* (Benn.) Benth. The family Marantaceae otherwise known as the sweet plater plant, is native to the western part of Africa, from sierra leone to Zaria. It is a large flowering herb with rhizomatous rootstock, growing about 4 m in height. It is a natural source of thaumatin, a low-calorie sweetener and flavor modifier protein. The leaves are ovate-elliptic, large, and papery.Phytochemicals seen in *Thaumatococcus daniellii* includes; Alkaloids, Flavonoid, Tannins, Saponin, and Cardiac glycoside [13].

Nigeria researchers have demonstrated how the Moi moi leaf plant (thaumatocDanielleelli) protects against complication of diabetes such as kidney and liver failure. A study was carried out to investigate the hepatoprotective role of T. danielli in carbon tetrachloride-induced hepatotoxicity in rats and histology and ultrastructure results confirmed that pretreatment with Danielle dose-dependently reduced hepatocellular necrosis [14]. The main aim of this research is to evaluate the haematological and histological assessment of the effect of *Thaumatococcus daniellii* methanolic leaf extract on lead induced toxicity in adult wistar rat.

2. Material And Method

Procurement of Lead

The lead was purchased as lead acetate Manufactured by BDH chemical Ltd Poole England from Effective chemical store Yenagoa, Bayelsa state.

Sources of *Thaumtococcus daniellii*

Fresh leaves of *Thaumatococcus daniellii* were obtained from the Amassoma community of Southern Ijaw Local Government Area of Bayelsa state. The leaves were identified by Dr. Alade (pharmacist). The leaves were shade-dried at room temperature to a constant weight and thereafter ground to powder packed in a dark polythene bags and stored in a desiccator for subsequent uses. About 200g of the powdered sample was dissolved in one liter methanol at room temperature for 72hours. This was then

filtered using Whatman NO.1 filter paper and the filtrate was transferred into a rotary evaporator at 40 degree Celsius. The residue obtained was further dried in a water bath at 37 degree Celsius and stored in a refrigerator at 4 degree Celsius.

Animal Housing

Thirty (30) albino Wistar rats weighing between 100g ± 2.5 – 142g ± 3.5 were used for this study. These animals were obtained from the animal house of the University of Port Harcourt, Rivers State, Nigeria. They were housed under a standard condition of temperature (27 ± 20^0C) with twelve hours of light and dark periodicity. These animals were housed in clean gauzed cages in groups and fed on standard feed pellets (Guinea feed Nigeria plc) and clean water ad libitum throughout the duration of the study. Acclimatization was for two weeks. Animals were handled in the study according to institutional guidelines for experiments involving the use of animals.

Experimental Design

The animals were weighed and divided into five groups. The duration of this study was for Twenty-one (21) days, the animals were allowed to acclimatize for two weeks. After the acclimatization period, thirty (30) young female rats were randomly divided into five groups, A, B, C, D, and E (6 rats in each group).

Group A(control): Rats were administered orally with pelleted growers mash (feed) and water throughout the experiment (21 days), **Group B**: Rats were administered orally with a lead only (150mg/kg) and given pelleted growers mashes (feed) and water for 21days. **Group C**: Rats were administered orally with lead (150mg/kg) and *Thaumatococcus daniellii* leaf extract (200mg/kg), pelleted growers mash (feed), and water throughout the experiment (21 days). **Group D**: Rats were administered orally with 200mg/kg of *Thaumatocccoccus danellii* leaf extract and 70mg/kg of lead for 21 days, and they were also given pelleted growers mash (feed and water throughout the experiment (21 days). **Group E**: Rats were administered orally with 200mg/kg *Thaumatococcus daniellii* extract and given palleted growers mash and water for 21 days.

Collection of Blood Sample

All experimental rats were sacrificed at the end of the experiment by anesthetizing them with diethyl-ether, Collection of blood Samples was done using 5ml syringes with 21G needles. The samples were collected from the animals through the cardiac puncture into pre-labeled Ethylenediamine tetraacetate (EDTA) vials and gently agitated to ensure EDTA is spread uniformly after which the samples were immediately used for measurement of hematological parameters like the hemoglobin concentration, Packed Cell Volume (PCV), differential white blood cells, and total WBC.

Measurement of Haematological Parameters

Blood samples collected from the experimental animals were introduced into appropriately labeled EDTA containers. The hematological evaluation was conducted on the samples.[15]

Determination of Lead

An automatic absorption spectrophotometric method using a chelation-extraction procedure was used for blood lead level determination.

Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM). Computer software, SPSS (version 17.0) was used for data analysis. Statistical measures used were One Way Analysis of Variance (ANOVA) along with post hoc multiple comparison test (least square difference procedure). Values of $P<0.05$ was the criterion for statistical significance.

3. RESULT

Histology Photomicrograph Plates

Plate 4.1 Shows the morphology of the liver after staining with hematoxylin and eosin and viewed at x400 magnification. Sections showed normal slides with the central vein (CV). Hepatocytes with intact sinusoidal space (S) are consistent with normal liver histology.

PLATE 4.2: Shows the morphology of the liver after the administration of the various treatments for 21 days. The slide shows congestion of the central vein (CV), areas of focal necrosis (arrow), and occluded sinusoidal space (S) with the presence of Kupffer cells (K) (X10) (X40) H&E.

PLATE 4.3: Shows the morphology of the liver after the administration of the various treatments for 21 days. The slide shows normal morphology of the liver, central vein (CV), hepatocytes (H) with intact sinusoidal space (S), and Kupffer cells (K) (X10)(X40)H&E

PLATE 4.4: Shows the morphology of the liver after the administration of the various treatments for 21 days. The slide shows normal morphology of the liver, central vein (CV), hepatocytes (H) with intact sinusoidal space (S), and a few Kupffer cells (K) (X10)(X40)H&E

Plate 4.5 Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows normal morphology of the liver, central vein (CV), hepatocytes with intact sinusoidal spaces (S) (X40)

Plates

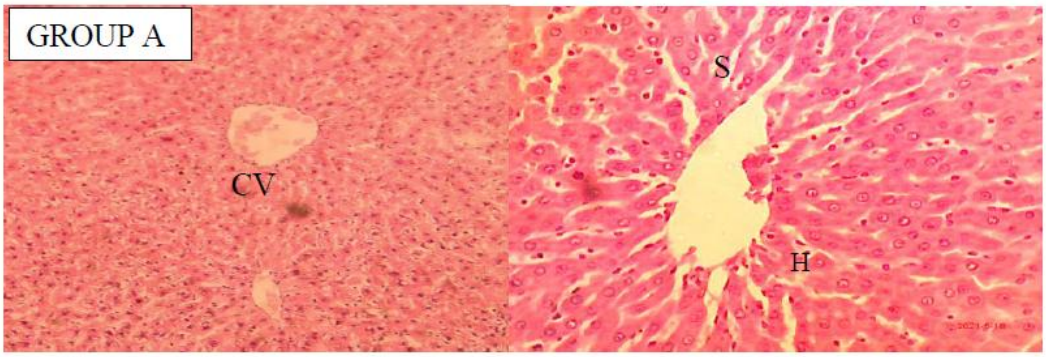
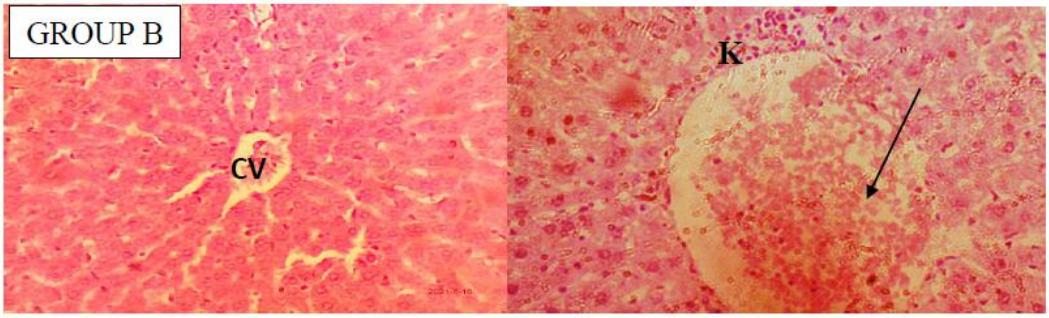


PLATE4.1. *Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows normal morphology of the liver, central vein (CV), hepatocytes (H) with intact sinusoidal space (S) (X10)(X40)H&E*



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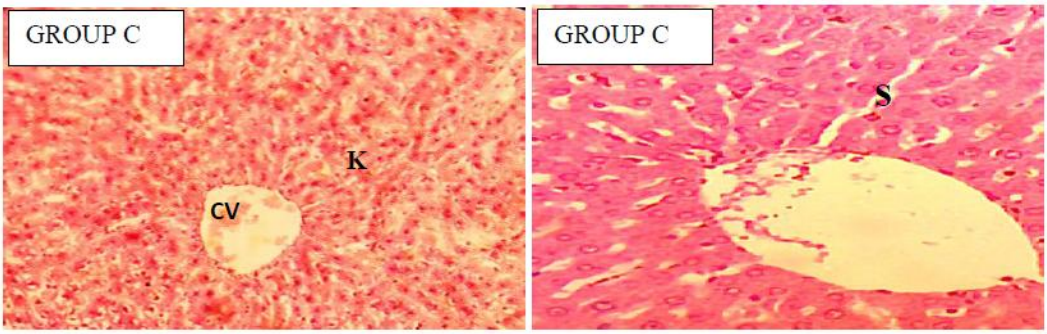

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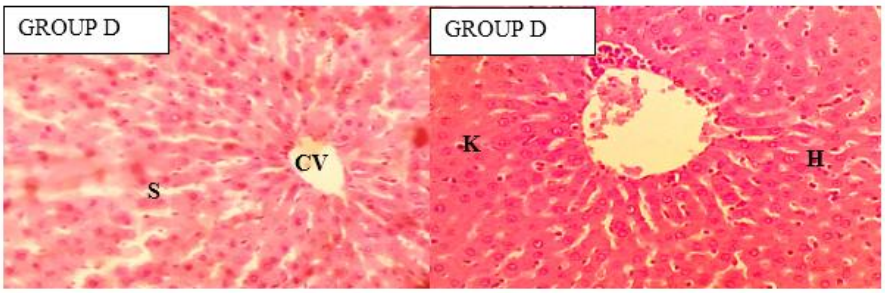

PLATE4.4. *Shows the morphology of the liver after the administration of the various treatments for 21 days. The slide shows normal morphology of the liver, central vein (CV), hepatocytes (H) with intact sinusoidal space (S), and a few Kupffer cells (K) (X10)(X40)H&E*

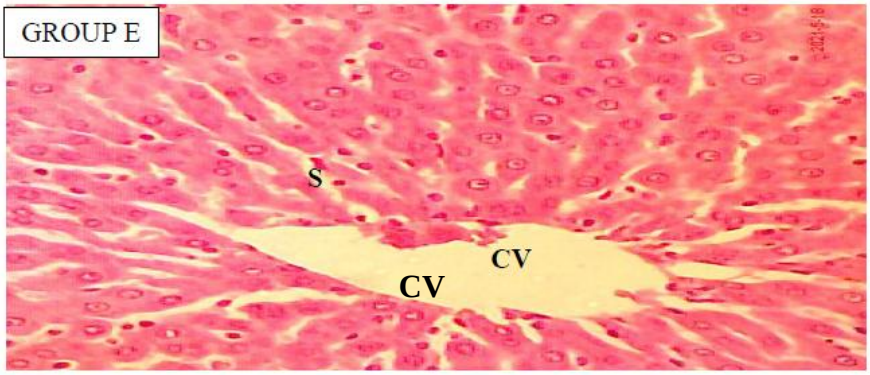

PLATE4.5. *Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows normal morphology of the liver, central vein (CV), hepatocytes with intact sinusoidal spaces (S) (X40)*

Histology Photomicrograph Plates

Plate 4.1 Shows the Morphology of the kidney after the administration of the various treatments for 21 days. Slide shows normal morphology of the kidney,glomeruli (G),distal tubules(DT).(X40).

PLATE 4.2: Shows the Morphology of the kidney after the administration of the various treatments for 21 days. Slide shows increased size of glomeruli (G) with normal morphology of distal tubules (DT) (X10) (X40) H&E.

PLATE 4.3: Shows the Morphology of the kidney after the administration of the various treatments for 21 days. The slide shows normal glomeruli (G), distal tubules (DT), and presence of mesangial cells(MS). (X40) H&E

PLATE 4.4: Shows the Morphology of the kidney after the administration of the various treatments for 21 days. The slide shows normal glomeruli (G) with presence of mesangial cells(MS).(x10)(X40) H&E

Plate 4.5 Shows the Morphology of the kidney after the administration of the various treatments for 21 days. Slide shows the normal morphology of the kidney, glomeruli (G), and distal tubules(DT). (X40)

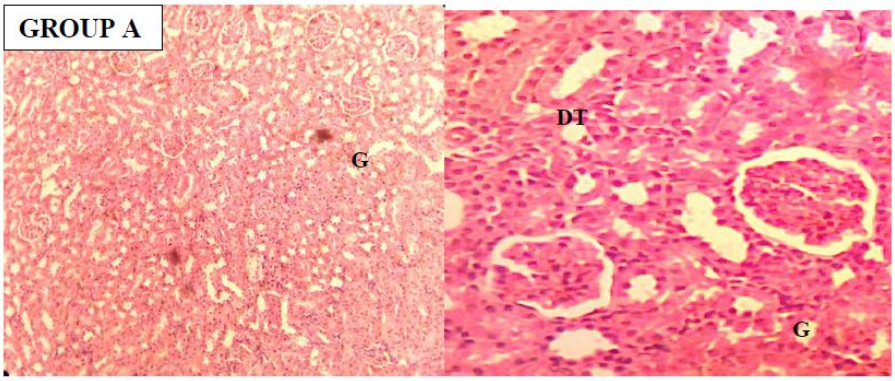

PLATE4.1. *Shows the Morphology of the kidney after the administration of the various treatments for 21 days. The slide shows the normal morphology of the kidney, glomeruli (G), and distal tubules(DT). (X40)*

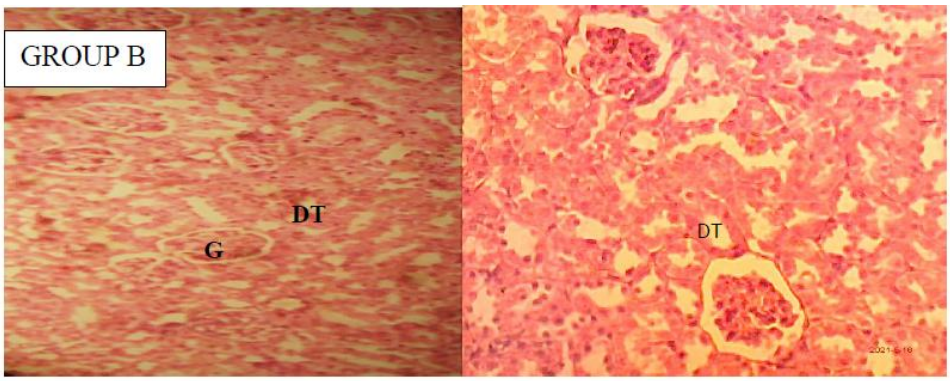

PLATE 4.2. *Shows the Morphology of the kidney after the administration of the various treatments for 21 days. Slide shows increased size of glomeruli (G) with normal morphology of distal tubules (DT) (X10) (X40) H&E.*

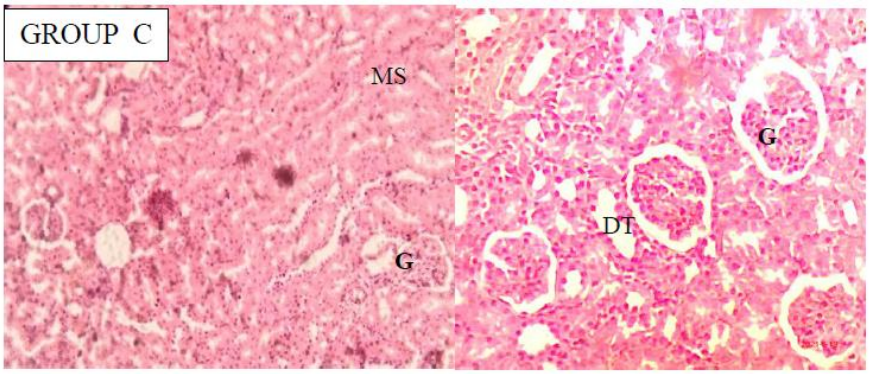

PLATE 4.3. *Shows the Morphology of the kidney after the administration of the various treatments for 21 days. The slide shows normal glomeruli (G), distal tubules (DT), and the presence of mesangial cells(MS). (X40) H&E*

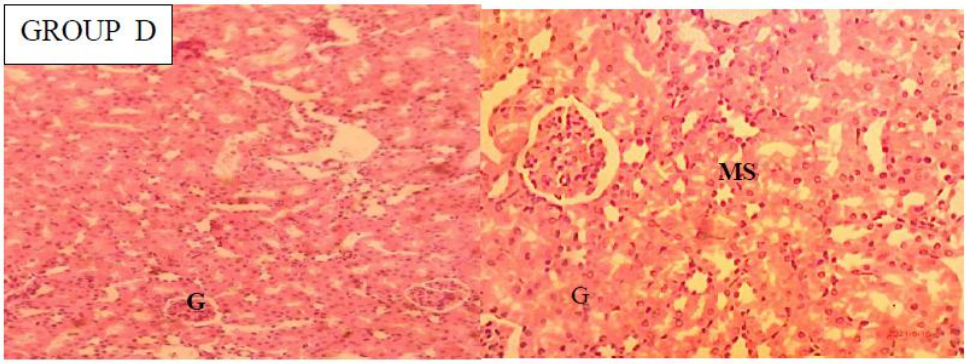

PLATE4.4. *Shows the Morphology of the kidney after the administration of the various treatments for 21 days. The slide shows normal glomeruli (G) with the presence of mesangial cells(MS).(x10)(X40) H&E*

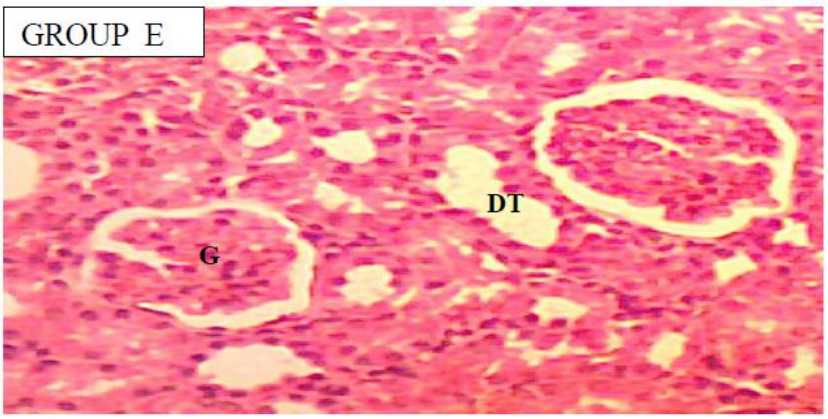

PLATE4.5. *Shows the Morphology of the kidney after the administration of the various treatments for 21 days. The slide shows the normal morphology of the kidney, glomeruli (G), and distal tubules(DT). (X40)*

Table4.1. *mean ± standard deviation (SD) of the biochemical parameters among the groups*

Parameters	Control Group A	Group B	Group C	Group D	Group E
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
AST	502.50± 102.53	342.50±130.82	305.00±14.14	585.00±261.663	440.00±35.36
ALT	252.50±45.96	200.00±91.92	144.00±12.73	362.50±144.96	150.00±0.00
T. PROTEIN	93.00±21.21	75.50±0.71	76.50±4.95	74.00±2.83	74.00±4.24
ALBUMIN	45.50±7.78	48.50±2.12	43.50±0.71	45.50±0.71	41.00±2.82
T. BILIRUBIN	22.00±4.24	21.00±9.89	25.50±7.78	16.50±2.12	20.00±2.82
C. BILIRUBIN	7.30±1.56	4.00±0.281	5.05±0.35	4.75±0.91	4.75±3.32

KEYS;

AST: ASPARTATE TRANSAMINASE

ALT: ALANINE AMINOTRANSFERASE

T. PROTEIN: TOTAL PROTEIN

T. BILIRBIN: TOTAL BILIRUBIN

C.ALBUMIN: CONGUGATED BILIRUBIN

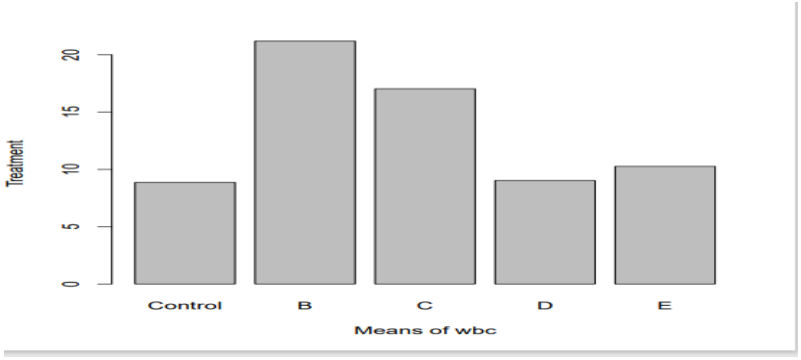

Figure4.1. *Bar chart of variance for White Blood cells among the groups.*

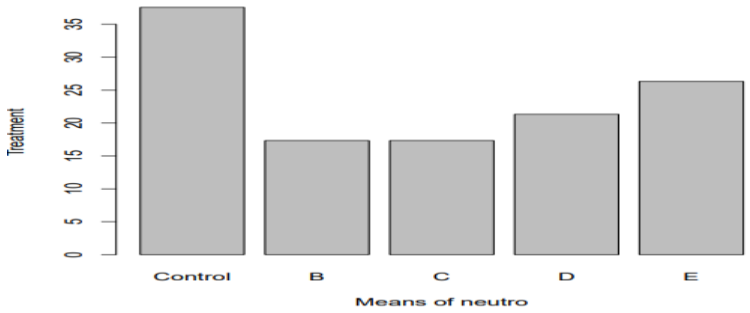

Figure4.2. *Bar chart of variance for Neutrophil among the groups*

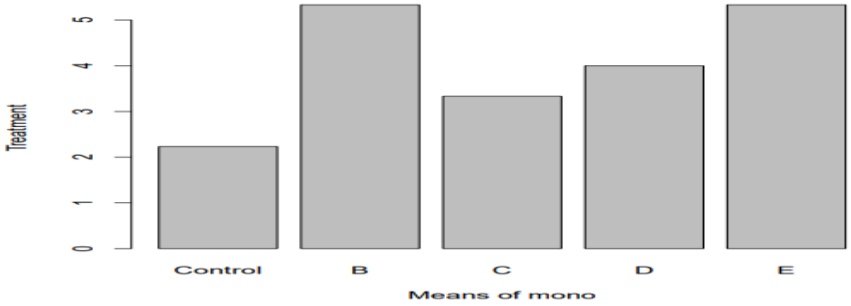

Figure4.3. *Bar chart of variance for monocyte among the groups*

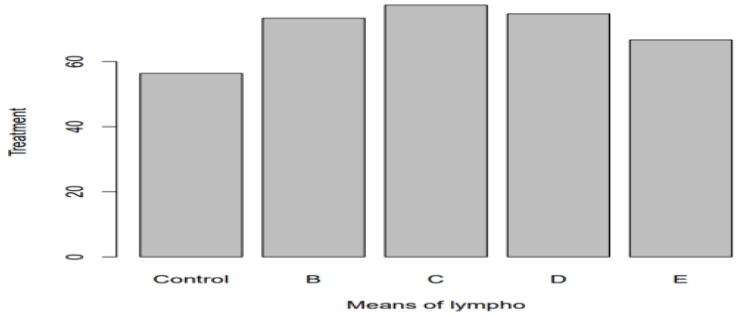

Figure4.4. *Bar chart of variance for lymphocytes among the groups*

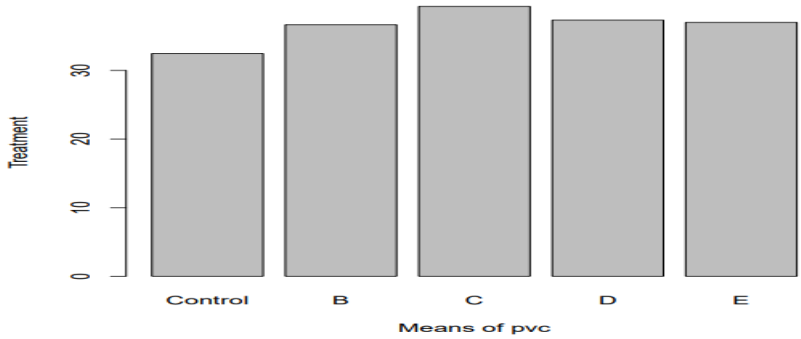

Figure4.5. *Bar chart of variance for Packed Cell Volume among the groups*

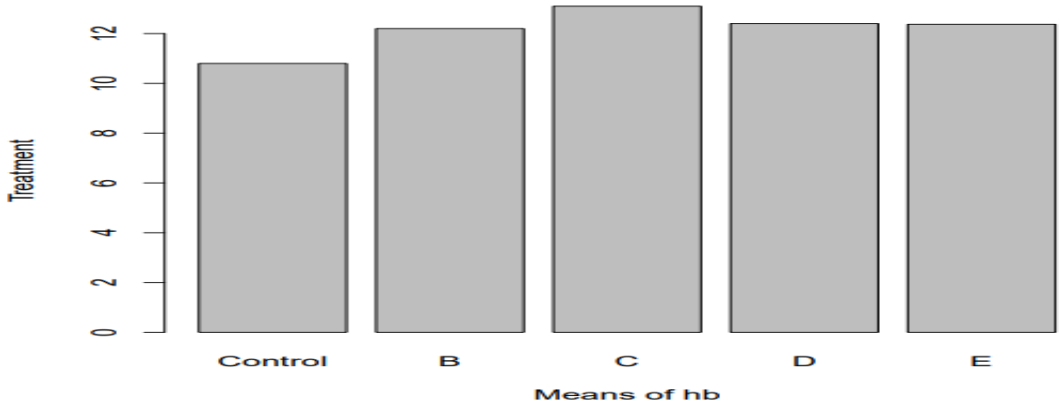

Figure4.6. *Bar chart of variance for Haemoglobin concentration among the groups*

Table 4.2. *Assessment of lead (Pb) in the samples in ppm (parts per million)*

Results obtained in the samples in ppm or mg/kg

A1	A2	B1	B2	C1	C2	D1	D2	E1	E2
0.1045	0.1842	2.0424	2.2367	0.7462	0.7364	0.4372	0.4334	0.1931	0.1263

Table4.3. *p-values of the biochemical parameters among the groups*

Parameters	**Group B**	**Group C**	**Group D**	**Group E**
AST	1.361	2.699	-0.415	0.815
ALT	0.722	3.217	-1.023	3.154
T. PROTEIN	1.166	1.071	1.256	1.242
ALBUMIN	0.526	0.362	0.000	0.769
T. BILIRUBIN	0.131	-0.559	1.640	0.555
CONG.BIL	2.952	1.995	1.996	0.983

4. DISCUSSION

The findings from this study on the hematological assessment show that Group B (rats induced with a lead only) have a significant difference in Hb, PCV, WBC Neutrophil, Lymphocytes, monocytes Eosinophil, and Basophil (P< 0.05). Group C (rats induced with lead and administered with *Thaumatococcus daniellii)* and Group D (rat administered with *Thaumatococcus daniellii* leaf extract and induced with a low dose of lead) show a slight significant effect in PCV, Hb, WBC, Neutrophil, lymphocyte, Monocytes, Eosinophil, and Basophil. Group E rats treated with *Thaumatococcus daniellii* leaf extract shows no significant difference in Hb, PCV, WBC, Neutrophil, Lymphocytes Monocytes, Eosinophil, and Basophil (P>0.05) as compared with the control.

Thaumatococcus daniellii leaf (Moi-Moi leaf) has been shown in other studies to have high nutritional value and potential medicinal uses and could serve as nutritious vegetables in addition to wrapping processed foods[16]. Studies have also shown that treatment with lead acetate at low doses hurts experimental animals including hematological alterations[17]. Gani *et al.*, (2017) in their study report a significant decrease in red blood cells, PCV, WBC, and Haemoglobin. This could be a result of the dose administered and the duration of the research. He administered 6mg of lead for 56days as compared to this study where we administered 150mg for 21 days thus the above result[17]. There was also a significant difference in both initial and final weight (p<0.05) of rats induced with a lead only. Adedosu *et al*., (2017) also reported that the leaf extract possesses anti-anemic and anti-oxidant properties when they treated streptozotocin-induced diabetic rats with *Thaumatococcus daniellii* leaf extract[18]. This is also attributed to the leaf extract having anti-anemic and anti-oxidant properties that were able to moderate hematopoiesis thus after the effect of lead toxicity on hemopoiesis, the leaf extract was able to ameliorate the hematological disorder[18]. Findings from Oke *et al*., (2016) revealed that the leaf possesses phytochemicals with the potential anti-oxidant activity,[19] and anti-oxidant is known to act as the first line of defense in the body against free radical-induced oxidative cell damage[20]. Some anti-oxidants could act independently or as a network to stabilize free radicals, pro-oxidant metals, and termite deleterious chain reactions elicited by free radicals [21]. While dietary anti-oxidants act by controlling free radical levels with a corresponding decrease in the symptoms of oxidative stress [22].

Based on the above results, the significant effect observed in the hematological parameters in groups C and D could also be attributed to the low quantity of lead found in the animal at the end of administration as seen in table 4.4 above.[23]. This agrees with the finding of Adu *et al*., 2021 in which there was no significant change (p>0.05) in Hb and PCV[24]. This also agrees with the findings of Ogoloma *et al*., 2017 in which there was no significant effect (p>0.05) on Hb and PCV[25].

The Plate labeled 4.1 -4.5 Shows the morphology of the liver after staining with hematoxylin and eosin and viewed at x400 magnification. The plates labeled 4.1-4.5: Show the morphology of the liver after the administration of the various treatments for 21 days. The slide labeled group A represents the animals in the control group which were given feed and water only Sections showed normal slides with the central vein (CV) and Hepatocytes with intact sinusoidal space consistent with normal liver histology. Those labeled group B are those to which lead was administered (1mg/kg), feed and water slide shows congestion of the central vein area of focal necrosis, occluded sinusoidal space with the presence of kupffer cells. The group labeled C were the animals given the extract (thaumatococcus danielli) (0.3mg/kg) lead(1mg/kg), feed, and water, slide shows normal morphology of the liver central vein, hepatocytes with intact sinusoidal space, and few kupffer cells. The group labeled D represents animals that were fed with thaumatococcus (0.9mg/kg), lead (1mg/kg), feed, and water slide shows normal morphology of the liver central vein, hepatocytes with intact sinusoidal space, and few kupffer cells. Lastly, the slide labeled E represents animals that were feed thaumatococcus Danielle (0.9mg/kg) slide shows normal morphology of the liver central vein, hepatocytes with intact sinusoidal space, and few kupffer cells. The various photomicrograph of the liver sections in plates 4.2(group B) agree however in the study carried out by Ali Sawsan *et al.,* 2018, according to their study, lead caused a significant increase in blood lead level (BILL) and serum malondialdehyde(MDA) concentration in rats treated with lead alone as compared to the normal control and this result may be caused by the effect of lead on the liver structure damaged of hepatocyte and disturbance in liver secretion[26]. They showed that oxidative stress is an important mechanism of lead-induced toxicity, imbalance, and removal of reactive oxygen species in the cellular structure causing damage to the membrane, membrane lipid, and protein [27][28]. Thaumatococcus danelli has antioxidant properties, research

works on ethnobotanical studies demonstrate the use of these plants in the management of diabetes [29]. Diabetes is generally believed to be accompanied by oxidative stress. This oxidative stress is associated with the pathogenesis of several diseases, including renal failure [27].In addition gas chromatography-mass spectrometry (GC-MC) 50 characterization of n-hexane, ethylacetate and methanol extracts of T. Danielle leaves identified; thirteen and fifteen compounds, with tetracontane (28.76%) and L-ascorbic acid (15.07%); hexadecanoic acid (21.62%) and y-sitosterol (11,06%); and naphthalene-1,2,3,5,6,7,8,8a-octahedron-1,8a-dimethyl-7-(1methyl phenyl)-, 1R[1.alpha.,7.beta.,8a.alpha.] (26.90%) and hexadecanoic acid (12.60%) being the major compounds respectively. The GC-MC analysis revealed various peaks of bioactive compounds which are the antioxidant [30].

5. CONCLUSION

The findings from this study indicate that the treatment of lead-induced toxicity with *Thaumatococcus daniellii* methanolic leaf extract has a significant effect on the hematological parameters at the dose administered (200mg) and therefore the leaf can be used as a replacement for synthetic additives in the food industry since it possesses anti-oxidant and anti-anemic properties. This suggests that it may play a critical medicinal and nutritional role and probably be a safer alternative to polyethylene paper and aluminum foil wraps; hence justifying its local usage as a food wrapper.

RECOMMENDATION

1. Detailed study on the effect of *Thaumatococcus danielli* on biological parameters and its antitoxic effect on lead poison
2. It is recommended that *Thaumatococcus danielli* leaf should be used as a food wrapper because it may introduce Phyto-oxidants into food processed and packaged with it due to its presumed safety and several therapeutic applications.
3. Further research should be carried out on how to use *Thaumatococcus daniellii* leaf effectively as a food wrapper.

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