e-ISSN :3027-2068:Print ISSN:3026-9830

***Int. J.Bio. Sc. Mol. Res. April* 2023 Vol.1(2)98 -116**

Ameliorative potential of Chloroform and Ethanolic Extract of *Whitfieldia lateritia* Hook. leaves and Chemiron(supplement) on some Biochemical Indices and Hepatic Histology of Phenylhydrazine-induced Anaemic Rats

[*] Okorie,U.C [1], Ugochukwu, A.O [2] ., Elem ,I [3]., Otu-Christian,G [4]., Ogunwa ,S.C [5]., [1] Ezennaya, C.F [6]., Igwe, D.O [7]., Igbokwe, S.O[8]., Nweke, T.O [9]. ,Ebe , I.T. [10] and Chukwuma,C. J [11].,

[1,3,4,5,6,8,9,10]Department of Biochemistry, Alex-Ekwueme Federal University Ndufu Alike, Abakaliki, Ebonyi State, Nigeria

[2]Animal Health and Production Technology, Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria

[4]Science Laboratory Technology Department, Akanu Ibiam Federal Polytechnic, Uwana, Afikpo, Ebonyi State, Nigeria.

[7,11] Department of Chemistry, Alex-Ekwueme Federal University Ndufu Alike, Abakaliki, Ebonyi State, Nigeria

***Corresponding author**: uchechukwuc2002@yahoo.com

Abstract

Background: Anaemia is a major health challenge in developing countries where access to quality nutrients and medicare remain worrisome. Increasing efforts in identifying medicinal plants with anti-anaemic potential, especially, in developing country like Nigeria led to the report on the 'ameliorative potential of chloroform and ethanolic extract of *W. lateritia* leaves on some biochemical indices and hepatic histology of phenylhydrazine (PHZ)-induced anaemic male albino rats. **Methododology:**The phytochemical screening of the extracts was carried out following standard procedures. Forty-five (45) male albino rats weighing 97 - 136grams were purchased and grouped into nine (9) of five rats each. G1 was the control,while G2 to G9 were induced using 4mg/kg body weight of 2-phenylhydrazine (PHZ). G2-G7 were treated with different doses of chloroform and ethanolic extracts of *W.lateritia* leaves and chemiron tablets, while G8 and G9 were negative and baseline control, respectively. Biochemical assays and liver histology were carried out following standard protocol.**Results:** Results of the phytochemical screening showed flavonoids, alkaloid, tannin and phenols in the ethanolic extracts of *W. lateritia* leaves , while steroid, alkaloid and resin were observed in the chloroform extracts of the *W. lateritia* leaves. The body weight gain was significantly increased in the test groups compared to the normal control(p<0.05). Liver alteration was restored in G2, G5 and G7, as compared with the control. Hemoglobin and red blood cell were restored from day 14 in group 2 treated with 50 mg/kg bd w chloroform extract of the *W. lateritia* leaf extract.**Conclusion:**The study showed that the chloroform extract of *W.lateritia* leaves had greater potency for the amelioration of the haematological and hepatic alterations

Keywords: anaemia, chloroform, haemoglobin, spectrophotometer and ketone

1.Introduction.

Plants have been used since ancient years in folklore medicine to combat different ailments, especially, in developing countries where access to quality food and health care services have a threat to the advancement of quality of life and general well-being of the populace. The

application of plant materials for the management of health problems is due to the presence of nutritional and non-nutritional components which contribute immensely to the general well-being of both humans and animals. The health benefits of plants stem from the ability of the components (e.g alkaloids, phenols, steroids, vitamins, etc) to provide antioxidant, antimicrobial, anti-anemic, hypoglycemic and hypocholesterolemic activities (Aja *et al*., 2016). The contributions of various plant parts (roots, stem, leaves, flower, and seeds) to the maintenance of cellular health has been reported (Ohshima *et al*., 2006).

Anaemia (a disease condition in which the body does not have adequate healthy red blood cells (RBCs) or hemoglobin (Hb) (Priyanka *et al.*, 2015), is one of the major health concerns in many tropical countries including Nigeria due to the prevalence of malaria and other parasitic infections which possibly can interfere with blood production (Toma *et al.*, 2015). Chemiron is one of the approved supplements for the treatment of anaemia; however, plants are currently used as an alternative therapeutic agent for anaemic treatment, because of their availability and affordability. In this study, the efficacy of Chemiron and the *W.lateritia* was evaluated. Reports on the efficacy of most plant extracts in the treatment of anaemia are common (Priyanka *et al.*, 2015; Sherry *et al*., 2016; Martha *et al.*, 2018). The quest for plants with high medicinal potency is one of the bases for the study on plants' therapeutic potentials; however, plants with fewer documentary records attract more attention.

Whitfieldia lateritia is one of the numerous plants that are under-studied and under-valued. It belongs to the family, *Acanthacea*, which has 10 species (Hutchusion and Dalziel, 1973). It is an evergreen plant that can easily be identified by the well-developed leaves arranged alternately along the stem (Aja *et al.*, 2016). It is used in folklore medicine for the treatment of anaemia and high fever (Aja *et al*., 2015). *W. laterita* leaves are rich in essential amino acids and vitamins such as vitamin B12 and folic acid which are important in the biosynthesis of haemoglobin (Okorie *et al*., 2020a). Although *W. laterita* leaf decoction is used in folklore medicine for the treatment of anemia (Okorie *et al*., 2020b), the use of other types of solvent extracts, and the scientific basis for the antianaemic potential have not been investigated. In line with this, the current study reported the ameliorative potential of chloroform and ethanolic extracts of *W. lateritia* leaves and Chemiron(supplement) on some biochemical indices and hepatic histology of phenylhydrazine-induced anaemic rats.

2. Materials and Methods

2.1 Collection of experimental leaves (*W. lateritia*)

The fresh leaves of the Whitfieldia lateritia plant were collected in August 2021 from a forest at Ishiagu Ivo L.G.A. Ebonyi State, Nigeria

2.2 Preparation of Experimental Leaves

The leaves of *Whitfieldia lateritia* were destalked, washed, and dried at room temperature with constant turning. The leaves were milled to powder and stored in an air-tight container.

2.3 Extraction of *W. lateritia*

Fifty (50) grams of dried powdered leaves of the *Whitfieldia lateritia* plant were extracted with 500 mL of ethanol per extraction, using a soxhlet extractor. The extract was concentrated to paste at 40 ^{o}C using a water bath

Percentage yield (%) yield $= \frac{weight\ of\ beaker+extract-weight\ of\ empty\ beaker}{\text{initial weight of sample used}}$ x 100

2.4 Column chromatography separation of the ethanol Extract

One (1) liter column was packed tightly with cotton wool and a silica gel, and held with a retort stand firmly. The column was soaked with ethanol to immerse the gel and tightly it in the column. The extract was added to the column followed by elution using the ethanol solvent, followed by chloroform. The extracts were collected separately and concentrated using the water bath

2. 5 Qualitative phytochemical analysis of the column chromatography extracts of the leaves

Qualitative tests of phenols, alkaloids, saponins, flavonoids, tannins, and steroids were carried out on the leaves using the method described by Usman *et al*. (2009).

2.6. Experimental Design

Thirty (45) male albino rats weighing 112-127g were obtained from the animal house of the Alex Ekwueme Federal University Ndufu Alike. The rats were randomly assigned into nine groups (G1-control, G2 -phenylhydazine (PHZ)+200mg/kg bd w chloroform extract of *W.lateritia*, G3- 50 mg/kg bd w chloroform extract of *W.lateritia*, G4- 200 mg/kg bd w ethanolic extract of *W.lateritia*, G5 -50 mg/kg bd w ethanolic extract of *W. lateritia*, G6- 200 mg/kg bd w Chemiron, G7- 50 mg/kg bd w Chemiron, G8-negative control, induced but not treated and G9- baseline, sacrificed on the 3rd day after the induction of anaemia) of five rats per group. The rats were allowed free access to feed and water at room temperature in metal cages and treatment was continued for 21 days. except group 9 (baseline control).

2.7 Induction of Anaemia

Rats were induced with anaemia through oral administration of 10 mg/kg body weight of Phenylhydrazine for two consecutive days. On the 3rd day, following the manifestation of symptoms of anaemia, the group G9 (baseline control) was sacrificed and a blood sample was collected into plain sample bottles through ocular puncture; the liver was collected and preserved in the formalin. Biochemical and histological assay were carried out to confirm the baseline value and liver status after induction of anaemia.

2.8 Clinical Signs/ Observation

Itching of nostrils, yellowish urine, erection of nostrils hairs, and whitish skin color was observed.

2.9 Collection of blood samples

After the 21st day, rats (G1, G2, G3, G4, G5, G6, G7 and G8) were subjected to fasting overnight; the final weight was taken before they were sacrificed. The blood sample was collected into plain sample bottles using ocular puncture for the analyses of haemotological parameters and liver diagnostic enzymes while the liver was collected, preserved in the formalin, and used for histological analysis.

2.10 Biochemical Analysis

2.11 Determination of Hematological Parameters

The hematological parameters were determined using the Mayamed hematology autoanalyzer. Analyses were performed on an automated hematology analyzer (Siemens Healthcare Diagnostics, Advia 120) using mouse-specific algorithms and parameters (Technicon H•1E Multi-Species Software, version 3.0, Siemens Healthcare Diagnostics). Parameters determined included white blood cell (WBC) count and differential blood cell (RBC) count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count.

2.12 Determination of liver enzymes

The activities of Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were determined in the plasma ,using a piccolo chemistry analyzer following the methods of Wróblewski and La Due (1956) and Karmen (1955) as modified by Bergmeyer *et al*. (1977).

2.13 Histological analysis of the tissue (Liver)

Histological analysis was processed following the method described by Okorie *et al*., 2020c

2.14 Statistical analysis.

Data were analyzed using the statistical package for social science. Differences in means among groups,were analyzed using one-way analysis of variance (ANOVA), followed by the Duncan multiple range test. The results were expressed as mean±standard deviation, and differences were considered statistically significant at (p <0.05).

Table 1: Phytochemical screening of ethanol and chloroform extracts of *Whitfieldia lateritia* leaves.

Phytochemical	Ethanol	Chloroform
Saponin	-	-
Flavonoid	++	-
Steroid	-	++
Alkaloid	+	++
Tannin	++	-
Phenols	+	-
Resin	-	+

3. Result and Discussion

Flavonoids, alkaloids, tannins, and phenols were present in the ethanolic extract, while steroids, alkaloids, and resins were obtained in the chloroform extract of the *W. lateritia* leaf extracts (Table 1). Previous reports had indicated that different solvents have different extraction capabilities and spectrum of solubility for phytoconstituents (Aswanida, 2015). Leaves of plants have always been a source of a wide array of secondary metabolites with potential pharmacological properties (Russell and Duthie, 2011). Polyphenolic compounds which had been rated to occur ubiquitously in foods of plant origin, have many beneficial health effects, due to their potential antioxidant, anti-inflammatory and cancer-preventive activities (Li *et al.*, 2014). The quality of phytochemicals present in both extracts of the *W.lateritia* leaves may explain the basis for its medicinal applications. Okorie *et al*. (2021), observed the presence of flavonoids, saponins, steroids cardiac glycosides, and tannin in the decoction extract of *W. lateritia*. Phenols are known as important plant constituents that protects the plants from oxidant damage and are used for wound healing. Alkaloid on the other hand has antipyretic and anti-inflammatory capacity (Awan, 2014) , and also mediates in erythropoiesis (Okokon *et al*., 2007

Tannins are polyphenols that can form complexes with metal ions and with macro-molecule such as proteins and polysaccharides. They generally have astringent properties and bitter taste; used medicinally as antidiarrheal, hemostatic, and anti-hemorrhoidal compounds, and antioxidant agents. Tannins can act as an antifungal agent (Renee, 2015), generally, it inhibits organism by inactivation of the enzymes responsible for microbial adhesions (Vinoth, 2011). Tannins reduce feed efficiency and weight gain in chicks (Dei *et al*., 2007). Polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans ingested up to 1.0 g daily from a diet rich in fruits and vegetables (Gopalakrishnan and Dayakumar, 2017). Just like tannins, high levels of saponins affect feed intake and reduce the growth rate in poultry due to the bitter and irritating taste of saponins (Ogbe, 2012). Excess saponins cause hypocholesterolemia by binding to the cholesterol making it unavailable for absorption. Saponins also have hemolytic activity against red blood cells, and can form saponin-protein complex which reduces protein digestibility (Soetan and Oyewole, 2009; Ogbe, 2012). Saponin has also been described by Ajiboye (2013) as one of the useful ingredients in cosmetics. Cardiac glycosides have been used traditionally as arrow poisons, abortifacients, emetics, diuretics, and heart tonics, and recent findings, on the cellular pharmacology of cardiac glycosides as they relate to the treatment of human cancer (Renjini *et al.*, 2017). They can inhibit the membrane-bound sodium and potassium pumps responsible for the sodium and potassium exchange, exerting a positive inotropic effect on the heart in cardiac failure. The steroid found in these leaves are of importance in pharmacy, due to its relationship with compounds such as sex hormones,the leaves of *W. lateritia* may be useful for expectant mothers or breastfeeding mothers to ensure their hormonal balance since steroid compounds could be a potent starting material for the biosynthesis of sex hormones (Ajiboye, 2013).

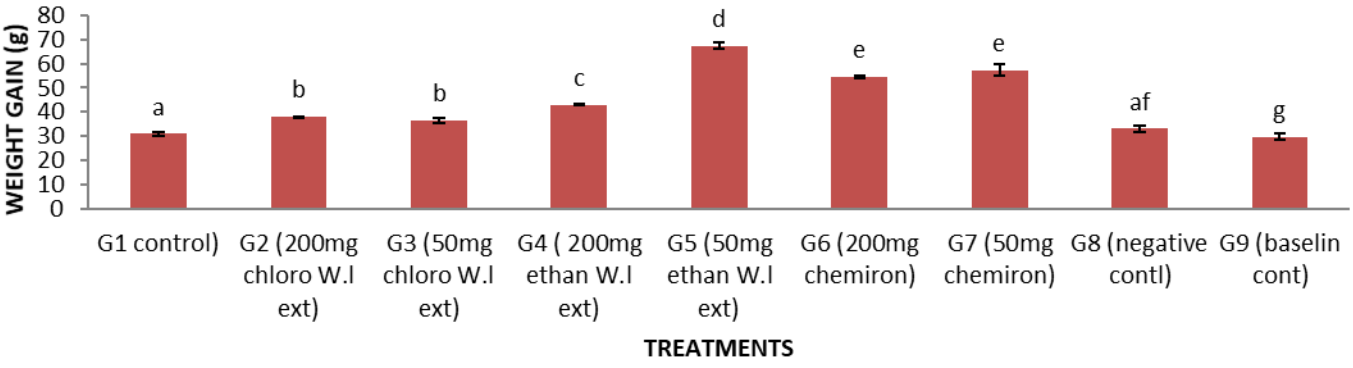

Figure 1. Effect chloroform w.lateritia leaf extract, ethanol W.lateritia leaf extract and chemiron on the Weight gain of PHZ-induced anaemic rats.

Mean ± SD; n=5, values on the bar with the same superscript are not significantly different (p>0.05). Key: mg =mg/kg bdw

The weight of the negative did not differed from the normal control, but the baseline control reduced (p<0.05), compared to the normal control. The body weight gain increased significantly (p<0.05), in the test groups compared, to the normal control (Figure 1). However, the groups treated with the 50 mg/kg bd wt ethanol were higher (p<0.05) than the groups treated with the chemiron. The observed highest weight gain in the 50 mg ethanol-treated group could be as a result of the ability of the extract to stimulate appetite, food absorption, and utilization or due to the presence of other active nutrients. Generally, the decreased weight observed in the baseline control was reversed by the chloroform extract and ethanolic extract of the *W.lateritia* leaf extract and the Chemiron.Toxicant like phenylhydrazine negatively alters the body weight of exposed rats (Kaware, 2013).The gradual appreciation in weight upon treatment could be, possibly through increased nutrient utilization and or contribution to nutrient availability for the tissues (Luke *et al*., 2013).

Anaemia is one of the global health issues, especially, in developing countries like Nigeria. It occurs as a result of a decrease in red blood cells. Table 2 shows that on day 7, day 14 and day 21 the WBC, RBC, PLT, LYM, and HCT were significantly increased (p<0.05), in the tests groups when compared with day 0 (baseline control- G9), while MCH, MCHC, and MON were significantly elevated at day 0 (p<0.05) , (p<0.05) ,when compared with the test groups at day 7, 14 an 21. The pattern of haematological alteration indicated that the blood indices (e.g. WBC, RBC, PLT, and HCT) which were severely decreased at the 0 (G9), began to show an incremental rise from day 7, but at day 14 and 21, the incremental elevation were not relatively different. The MCH, MCHC, and monocytes which were elevated (p<0.05) on day 0 (G9) decreased on day 7 in all the test groups ,while on day 14 and day 21, the decrease were also not different. Previous reports showed that PHZ could decrease haemoglobin levels, RBC (Red Blood Cell) count, and HCT (Hematocrit), whereas it increases the MCV (Mean Corpuscular

Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration (Singh *et al*., 2014). The lowest decrease observed in baseline control (G9), relative to the test groups, could be as a result of the effect of PHZ that caused a poor utilization of nutrients (Shakamaki *et al*.,2014). The elevation of the red blood cells and haemoglobin in the groups treated with chloroform and ethanolic extracts of the *W. lateritia* leaf, when compared to the Chemiron-treated groups, may suggest the presence of erythropoietic factors in both extracts. The work of Okorie *et al*.(2021), had shown that the *W.lateritia* leaf decoction possessed anti-anaemic potential. Alkaloid, which was found in the extract might be playing a role in the ameliorative activity of the extract, since Okokon *et al*.(2007) had reported that alkaloids could improve hematological indices. Through a process known as erythropoiesis, which takes place over the course of around seven days, committed stem cells that are developing transform into human erythrocytes.

Bone marrow may make up for the loss of many pints of blood each week if it is stimulated to its optimal activity, and given the necessary nutrients. The oxygen tension of arterial blood, which is typically brought on by the PHZ, a well-known hemolytic agent, has an impact on the rate of erythropoiesis. A vital part of the innate immune system, platelets are cell fragments that stop bleeding in broken blood arteries (Melva and Pasar, 2017). Haematocrit percentage is the ratio of erythrocytes to the total blood volume which is between 45-47%. MCHC in male rats aged 17 months or more, has been reported to be 35.1 g / dl , against 4.40 ± 0.91 noted in the baseline (G9) which was, however, reversed to normal after treatment. MCHC is often reduced in hemolytic anaemia or increased, in cases of massive intravascular hemolysis. Lymphocyte is part of the immune system that defends and acts to recognize antigens, which elicits the production of antibodies. The platelet, also known as thrombocyte, functions along with the coagulation factor to mediate during bleeding from blood vessel injury by clumping, thereby initiating a blood clot (Li *et al.*, 1999). Bone marrow is a primary lymphoid tissue and a major haematopoietic organ responsible for the production of about 500 billion blood cells daily in the form of Red blood cells, White blood cells, and Platelets (Adewoye *et al.*, 2013). The plants may contain materials that stimulate the bone marrow to produce more erythroid series that enhance the elevation of blood.

Figure 2. showed that the red blood cell and haemoglobin at day 0 were significantly decreased ($p<0.05$),but gradually increased as treatment commenced. The peak was attained at 14 weeks, with the 50 mg/kg bd w chloroform extract of the *W. lateritia* leaf showing the highest increase. Treatment with chemiron did not show a significant rise in the haemoglobin on days 7 and 14 when compared with the baseline control, day 0, until day 21 when treatment at 200 mg/kg bd w stimulated significant elevation. The level of red blood cells, which had drastically reduced at day 0, gradually increased as treatment progressed, but peaked at day 21 after receiving 50 mg/kg bd of the chloroform extract of the leaves of W lateritia. Due to inadequate dietary intake, anemia is frequent in developing nations like Nigeria (Kumar *et al.*, 2003). Red blood cell loss is the cause, and is usually treated with folic, vitamin B12, and iron supplementation, however, these drugs, sometimes, have side effects, such as hemochromatosis, and they are not always available or affordable to the sufferers, leading to dependence on herbal drugs (Kumar *et*

al.,et.,2003). Plant materials are known to have hemopoietic factors that have a direct influence on the production of blood in the bone marrow (Hye *et al.*, 2014). *W. lateritia* is being used in traditional medicine to treat anaemia in South-Eastern Nigeria (Aja *et al.*, 2016; Okorie *et al.*, 2020b). However, understanding the active ingredients and possible mechanisms responsible for the positive effects on anaemic rats demands further investigation.

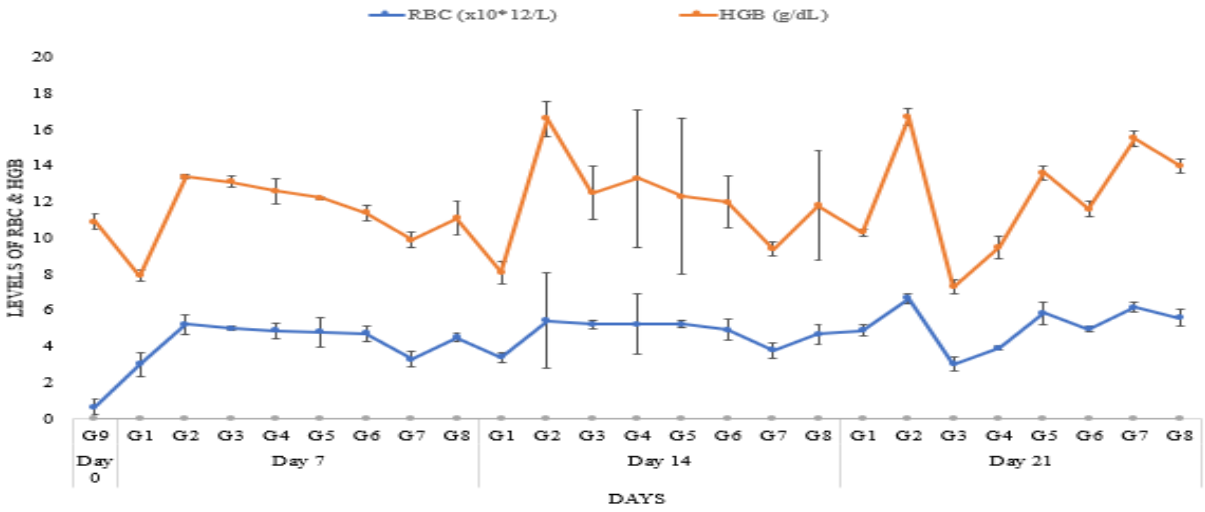

Figure 2: Change in RBC and HBG at day 0,mday 7, day 14 & day 21 in the rat groups treated with Chloroform, ethanol and chemiron

G1-control, G2 -PHZ+200mg/kg chloroform extract of *W.lateritia*, G3- 50 mg/kg bd w chloroform extract of *W.lateritia*, G4- 200 mg/kg bd w ethanol extract of *W.lateritia*, G5- 50 mg/kg bd w ethanol extract of *W. lateritia*, G6- 200 mg/kg bd w chemiron, G7- 50 mg/kg bd w chemiron, G8- negative control and G9- baseline control.

The liver plays a central role in animal metabolism, including synthesis, degradation, and detoxification. ALP was decreased ($p<0.05$) in the baseline control when compared to the normal control, but increased in the negative control compared to the normal control. However, AST and ALT were not significantly different ($p>0.05$), compared to the normal control. The activities of AST, ALT and ALP of the chloroform extract, ethanolic extract, and the chemiron treated groups did not vary significantly ($p>0.05$), from the normal control ,except G3, G4, G6, and G8 for ALP, but decreased significantly ($p<0.05$) from the baseline control (Figure 3). The observed non-significant variations of the activities of AST, ALT, and ALP of the chloroform extract, ethanolic extract, and the chemiron treated group when compared with the normal control, is an indication of hepatic healing of the liver alteration caused by the PHZ, which is evident in the baseline control and the negative control. The fact that the enzyme level was lower in the baseline control, shows that the enzymes might have been activated by alternate clearance systems, which would explain why it was lower.

Table 2. E**ffects of ethanol, chloroform extract *W. lateritia* leaf and chemiron on the hematological parameters of Phenylhydrazine-induced anaemic male albino rats.**

		WBC (x10*9/L)	LYM (%)	MON (**109/L**)	NEUT (%)	RBC (x10*12/L)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (x10*9/L)
Day0 (base)	G9	3.60 ± 0.41 a	81.00 ± 0.40 a	51.10 ± 0.31 a	13.90 ± 0.43 a	0.65 ± 0.41 a	10.90 ± 0.41 a	4.40 ± 0.91 a	68.7 ± 0.21 a	167.60 ± 0.71 a	247.70 ± 0.47 a	163.30 ± 64.05 a
Day 7	G1	5.10 ± 0.38 b	67.00 ± 0.01 b	4.90 ± 0.41 b	18.00 ± 0.21 b	3.00 ± 0.65 b	7.90 ± 0.31 b	24.00 ± 0.52 b	49.50 ± 0.82 b	18.90 ± 0.76 b	35.50 ± 0.23 b	563.00 ± 25.83 b
	G2	6.40 ± 0.45 b	64.80 ± 0.46 b	28.20 ± 0.01 c	27.00 ± 0.23 c	5.23 ± 0.55 c	13.40 ± 0.11 c	38.20 ± 0.12 c	73.20 ± 0.22 c	25.60 ± 0.32 c	35.00 ± 5.63 b	630.00 ± 49.49 c
	G3	6.30 ± 0.21 b	64.50 ± 0.22 b	8.10 ± 0.61 d	26.00 ± 0.41 c	5.00 ± 0.11 c	13.10 ± 0.31 c	38.00 ± 0.41 c	71.00 ± 2.41 c	23.00 ± 0.51 d	32.54 ± 3.75 c	628.00 ± 38.99 c
	G4	9.60 ± 0.01 c	70.40 ± 1.21 c	7.50 ± 0.22 d	22.10 ± 0.11 d	4.88 ± 0.43 d	12.60 ± 0.71 d	40.40 ± 0.91 d	82.80 ± 0.62 d	75.80 ± 0.21 e	31.10 ± 0.11 c	784.00 ± 33.98 d
	G5	9.40 ± 0.44 c	70.20 ± 0.49 c	7.30 ± 0.41 d	22.20 ± 0.45 d	4.80 ± 0.81 e	12.20 ± 0.11 d	39.80 ± 0.12 cd	82.00 ± 1.42 d	25.10 ± 0.31 e	31.00 ± 0.22 c	784.00 ± 33.95 d
	G6	6.30 ± 0.40 b	79.90 ± 0.43 d	7.40 ± 0.01 d	12.70 ± 0.41 e	4.72 ± 0.41 e	11.40 ± 0.41 e	33.20 ± 1.41 e	70.40 ± 2.41 e	24.10 ± 0.41 c	34.3 ± 0.41 d	643.00 ± 44.43 c
	G7	4.10 ± 0.21 d	84.1 ± 1.41 e	4.20 ± 0.40 b	9.46 ± 0.38 f	3.30 ± 0.41 b	9.90 ± 0.41 f	26.00 ± 0.41 f	72.00 ± 3.40 e	23.00 ± 0.41 d	33.10 ± 0.41 d	360.00 ± 50.74 e
	G8	5.60 ± 0.33 b	93.00 ± 2.50 f	3.30 ± 0.11 e	3.40 ± 0.42 g	4.50 ± 0.25 e	11.10 ± 0.91 e	31.00 ± 0.84 g	69.00 ±2.41 ef	24.00 ± 0,31 c	34.20 ± 0.11 d	901.00 ±10.85 f
Day 14	G1	6.00 ± 0.15 b	68.00 ± 0.36 b	5.70 ± 0.51 f	19.00 ± 0.21b	3.40 ± 0.29 b	8.10 ± 0.63 b	25.00 ± 0.25 b	50.10 ± 0.45 b	19.40 ± 0.35 b	38.10 ± 0.35 e	540.00 ± 0.35 b
	G2	3.90 ± 0.36 e	79.90 ± 14.76 d	6.30 ± 0.22 g	13.80 ± 3.80 e	5.44 ± 2.61 c	16.60 ± 0.99 f	39.90± 10.70 cd	73.50 ± 0.40 c	30.50 ± 6.34 f	35.16 ± 4.12 b	699.00 ± 17.68 c
	G3	5.60 ± 2.71 b	83.40 ± 17.68 e	5.50 ± 2.85 f	11.10 ± 4.20h	5.23 ± 0.22 c	12.50 ± 1.48 d	35.50 ± 0.79 h	67.90 ± 0.95 g	23.90 ± 0.50 d	35.20 ± 17.24 b	477.00 ± 6.96 g
	G4	6.00 ± 3.19 b	87.40 ± 17.68 g	4.50 ± 2.02 b	8.10 ± 3.67g	5.22 ± 1.67 c	13.30 ± 3.82 e	40.30 ± 13.58 cd	77.30 ± 2.55 h	26.40 ± 9.96 c	34.20 ± 3.48 d	722.00 ± 23.70 h
	G5	5.90 ± 0.22 b	88.20 ± 4.52 g	4.60 ± 0.79 b	7.20 ± 4.40h	5.22 ± 0.20 c	12.30 ± 4.30 d	34.90 ± 0.41 h	66.90 ± 1.55 g	23.58 ± 0.16 d	35.20 ± 0.86b	702.00 ± 46.10 h
	G6	4.40 ± 0.14 d	88.00 ± 3.31 g	5.00 ± 0.14 f	7.00 ± 1.49h	4.92 ± 0.58 e	12.00 ± 1.42 d	37.10 ± 2.42 c	75.50 ± 5.31 i	24.30 ± 0.47 c	32.30 ± 5.22 c	308.00 ± 27.07 e
	G7	4.40 ± 0.22 d	85.80 ± 4.90 e	4.50 ± 0.32 b	9.70 ± 0.22f	3.79 ± 0.42b	9.40 ± 0.41 b	27.50 ± 4.81 i	72.70 ± 6.50 c	24.80 ± 0.32 c	34.10 ± 4.70 d	365.00 ± 42.57 e
	G8	6.20 ± 0.38 b	93.00 ± 1.59 h	3.50 ± 1.87 e	3.5 ± 1.87i	4.67 ± 0.53e	11.80 ± 3.04 e	33.2 ± 0.45 j	71.20 ± 4.12 e	25.20 ± 3.60 c	35.50 ± 10.25 b	905.00 ± 8.63 f
Day 21	G1	6.40 ± 0.36 b	70.20 ± 0.23 c	7.30 ± 0.86 d	22.50 ± 0.35d	4.90 ± 0.32 e	10.30 ± 0.2 3f	26.30 ± 0.18 f	53.70 ± 0.38 i	21.00 ± 0.18 g	39.10 ± 0.79ei	861.40 ± 12.92 j
	G2	5.80 ± 0.25 b	80.00 ± 2.06 d	5.40 ± 0.29 f	14.60 ± 0.18k	6.67 ± 0.28 f	16.70 ± 0.43 f	48.60 ± 0.12 k	73.00 ± 0.76 c	25.00 ± 0.15 c	34.30 ± 0.41 d	828.00 ± 5.83 j
	G3	6.00 ± 0.25 b	84.00 ± 1.73 e	6.10 ± 0.43 g	9.90 ± 0.92f	3.02 ± 0.38 b	7.30 ± 0.36 b	19.80 ± 0.50 l	65.60 ± 1.24 g	24.10 ± 0.51 c	36.80 ± 0.60 f	560.00 ± 1.35 b
	G4	5.10 ± 0.36 b	77.50 ± 0.79 i	6.20 ± 0.78 g	16.30 ± 0.22l	3.93 ± 0.10 b	9.50 ± 0.64 b	28.00 ± 1.46 i	71.30 ± 0.96 e	24.10 ± 3.19 c	33.90 ± 2.95 dg	530.00 ± 28.76 b
	G5	8.90 ± 0.76 f	85.20 ± 0.87 e	5.30 ± 0.63 f	9.50 ± 1.60f	5.84 ± 0.61 c	13.60 ± 0.41 c	39.60 ± 0.41 cd	68.30 ± 1.41 a	23.20 ± 0.41 d	34.10 ± 0.41 d	947.00 ± 42.53 f
	G6	11.50 ± 0.22 g	80.20 ± 0.18 d	7.50 ± 0.82 d	12.30 ± 0.35e	4.97 ± 0.14 b	11.60 ± 0.41 e	37.80 ± 0.41 c	76.20 ± 0.41 h	23.30 ± 6.00 d	30.60 ± 0.42 c	609.00 ± 3.41 c
	G7	9.40 ± 0.32 h	80.80 ± 0.91 d	6.60 ± 0.47 g	12.60 ± 1.61e	6.18 ± 0.24 f	15.50 ± 0.41f	41.20 ± 0.41 cd	66.80 ± 0.41 g	25.00 ± 2.85 c	37.60 ± 0.41 e	801.00 ± 55.23 j
	G8	7.90 ± 0.47i	81.50 ± 0.53 a	6.70 ± 0.35 g	11.80 ± 0.63 h	5.60 ± 0.47 c	14.00 ± 0.41 g	38.50 ± 0.41 c	68.90 ± 0.41 a	25.00 ± 3.90 c	36.30 ± 0.41 f	950.00 ± 37.82 f

The mean ± SD, n =5; values with similar superscripts down the column are not significantly ($p>0.05$) different.Key: white blood cell (WBC), lymphocyte (LYM), monocyte (MON), neutrophil (NEU), red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet (PL

According to Zaher *et al*. (2011), non-parenchymal liver cells, including Kuffor cells (KCs) and endothelial cells , and some serum marker enzymes, such as AST and ALT, undergo endocytosis , hence, it cannot be ruled out that these clearance systems will start to up-regulate, which would explain the observed decline . The AST, ALT, and ALP activities of the test groups, G2 and G5, and G7, were interestingly similar to those of the control group, indicating that the cellular lesion/reduced KC activation was reversible. Apart from the liver, AST was also present in the heart, skeletal muscle, kidneys, brain, and red blood cells, but ALT had low concentrations in skeletal muscle and kidney, and was more specific for liver damage. In the liver, ALT is localized solely in the cellular cytoplasm, whereas AST is both cytosolic (20% of total activity) and mitochondrial (80% of total activity (Edoardo, 2005; Dufour *et al*., 2000).

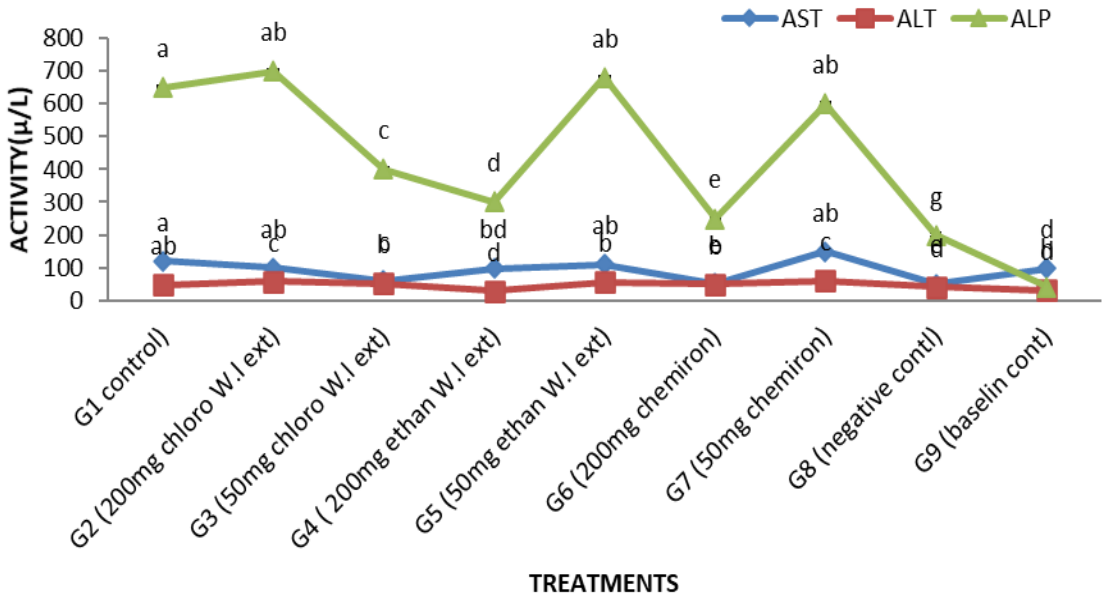

Figure 3. Effect of the chloroform, ethanol and chemiron on the liver enzymes

Histological analyses (Figures 4-12) revealed an effect on the hepatic tissue of the rats, with severe portal aggregate inflammation SPAI observed in the baseline control (Figure 12), and moderate healing in the test groups, whereas normal control showed normal hepatic architecture. This is consistent with previous reports that phenylhydrazine is toxic to exposed rats (Kaware, 2013), as evidenced in the baseline control (Figure 12), but gradual healing of the altered hepatic architecture was observed in all the test groups. The finding is in agreement with the previous report of Okorie *et al.* (2020c), who observed that *W. lateritia* leaf decoction was able to restore hepatic alteration in PHZ-induced anaemic cockerel. Although healing was observed in the test groups, none of the extracts displayed a higher degree of healing. Some plants have shown potency towards hepato-protection and hepato-restoration (Sumaia *et al*., 2019; Adewale *et al*., 2013), through different mechanisms ,including antioxidant activity.

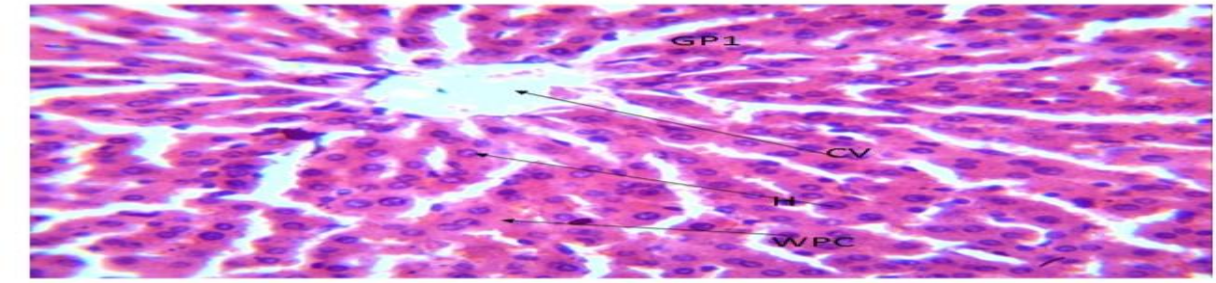

Figure 4: Micrograph of group 1 (G1) normal control section liver (x 400)(H/E) shows normal hepatic architecture with well-perfused cytoplasm (WPC) hepatocyte(H), and central vein (CV) cytoplasm (WPC) cytoplasm (WPC)

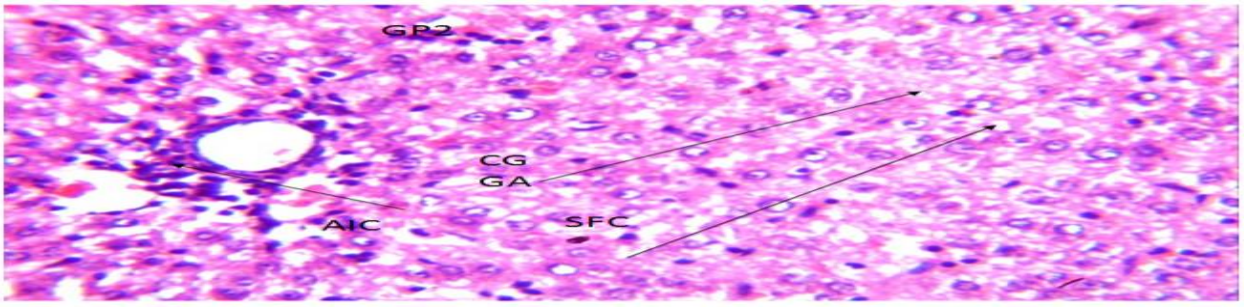

Figure 5: Photomicrograph of group 2 (G2) section of liver administered with phenylhydrazine and 200mg/kg bd w chloroform extract (x 400)(H/E) shows hepatic tissue with moderate congestion of the blood vessel (CBV), a mild aggregate of inflammation (AIC) around the central vein (AIC). And cytoplasmic ground glass appearance (CGGA).

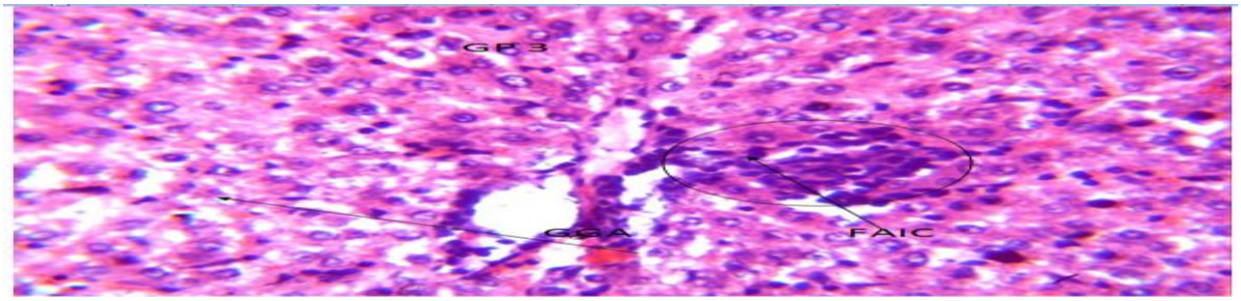

Figure 6: Photomicrograph of group 3 (G3) section of liver administered with phenylhydrazine and 50 mg/kg bd w chloroform extract *W. lateriacia* (x 400)(H/E) shows moderate healing with a focal aggregate of inflammation (AIC). And mild cytoplasmic ground glass appearance (CGGA).

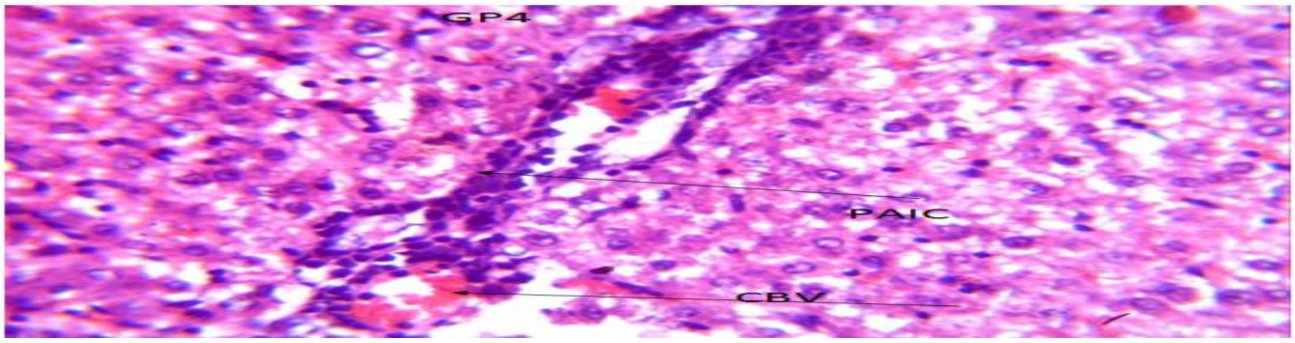

Figure 7: Photomicrograph of group 4 (G4) section of liver administered with phenylhydrazine and 200 mg/kg bd w ethanol extract W. lateritia (x 400)(H/E) shows moderate healing with intrahepatic inflammation (IHI) and congestion of blood vessel (CBV).

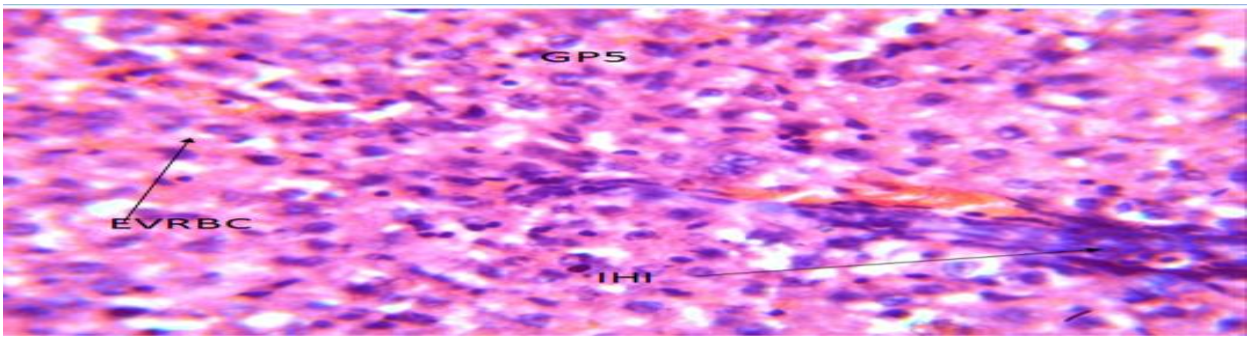

Figure 8: Photomicrograph of group 5 (G5) section of liver administered with phenylhydrazine and 50 mg/kg bd w ethanol extract W. latericia (x 400)(H/E) shows moderate healing with intrahepatic inflammation (IHI), extravasation of red blood cell (EVRBC) and cytoplasmic ground glass appearance (CGGA).

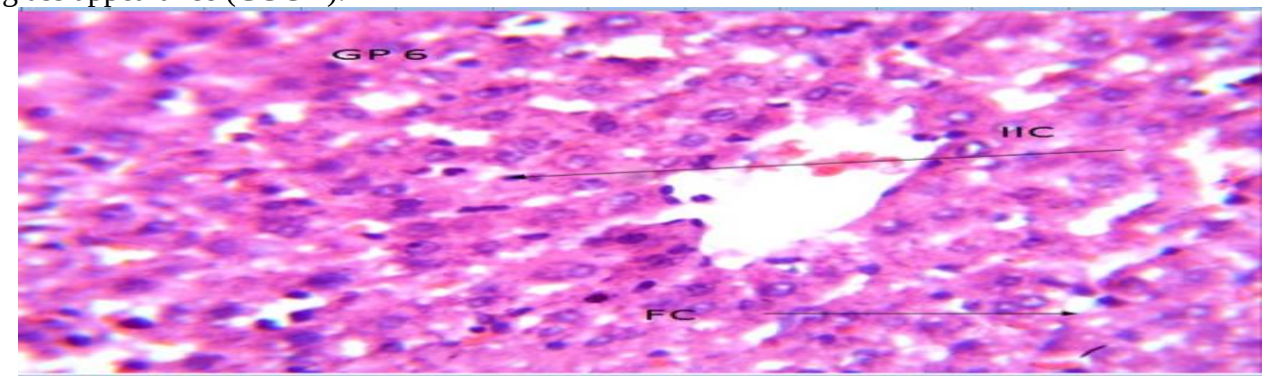

Figure 9: Photomicrograph of group 6 (G6) section of liver administered with phenylhydrazine and 200 mg/kg bd w chemiron (x 400)(H/E) shows moderate healing with mild infiltration of inflammatory cell and cytoplasmic ground glass appearance (CGGA) otherwise normal.

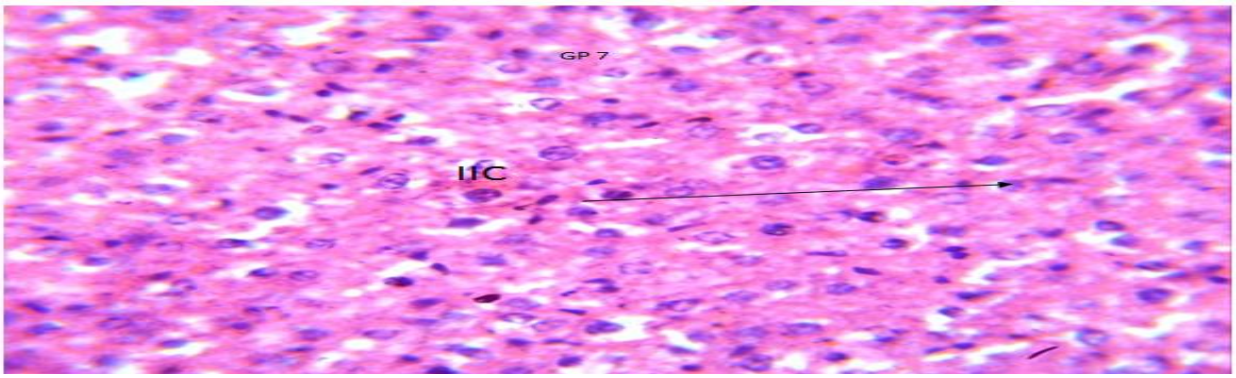

Figure 10: Photomicrograph of group 7 (G7) section of liver administered with 50 mg/kg bd w chemiron (H/E) shows moderate healing with mild infiltration of the inflammatory cell (IIC) otherwise normal.

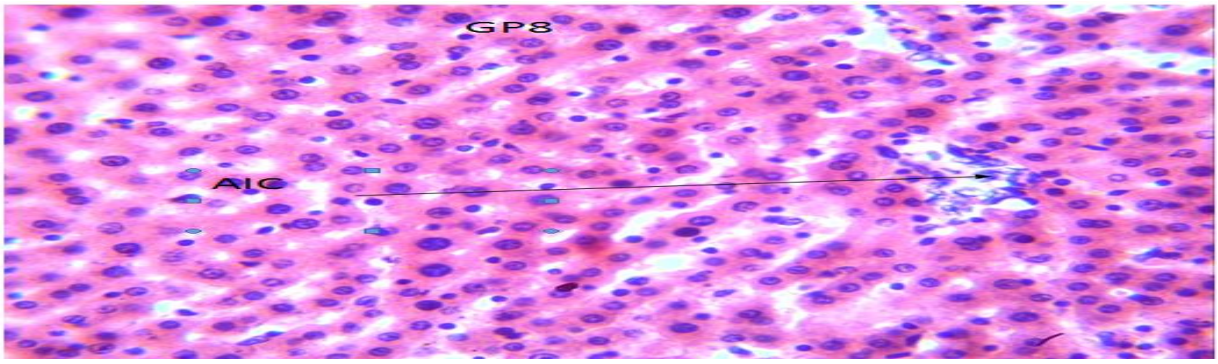

Figure 11: Photomicrograph of group 8 (G8) section of liver negative control (x 400)(H/E) shows moderate healing with a mild aggregate of the inflammatory cell (AIC) otherwise normal.

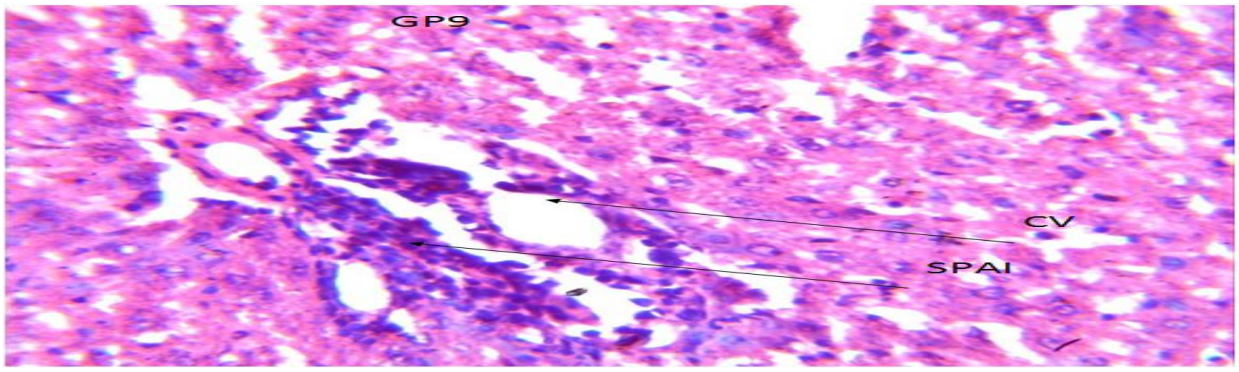

Figure 12: Photomicrograph of group 9 (G9) section of the liver (x 400)(H/E) induced with phenylhydrazine only shows the severe effect on the hepatic tissue with severe cirrhosis of the liver and severe portal aggregate inflammation, SPAI, (portal hepatitis).

Conclusion

The chloroform extract of *Whitfiedia lateritia* improved the haematological indices compared to the ethanolic extract and the chemiron (supplement). Both extracts exhibited hepato-restoration potential.

Reference

Aja, P. M., Ibekwe, E. U., Ekpono and Ugwu, P. C. (2015). Effect of Ethanol Extract of Cajanus cajan Leaf on Plasma Lipid Level in Albino rats. *International Journal of Current Research and Academic Review*, **3**(1): 161-167.

Adetutu, A and Olorunnisola,O.S(2013). Hepatoprotective Potential of Some Medicinal Plants against 2-Acetylaminoflourene-Induced Damage in Rat. *Journal of Toxicology*,2013:272097

Adewoye E.O, 1 Salami A.T and Jagun A. (2013). Full Length Research Paper Attenuation of Plasmodium Berghei berghei -induced Bone Marrow Suppression by Bark Extract and fractions of *Chrysophyllum albidum* on bone marrow response to haemolytic conditions in male albino mice *Tropical African Journal of Biomedical Ressearch*. 16: 47 – 53.

Aja, P.M., Nwuguru M.E., Okorie, U.C., Alum E.U. and OfforC.E.(2015) Effect of Decoction Extract of Whitfieldia lateritia on Lipid Profiles in Hypercholesterolemic Albino Rats . *Global Veterinaria*, 14 (3): 448-452.

Aja, P.M., Okorie, U.C., Ozougwu, V.E.O., Onya-Mmaghiri, E.A., Agu, K.A., & Nweke, O.L. (2016). Phytochemical and gas chromatography-mass spectrometric (GC-MS) analyses of *Whitfieldia lateritia* leaf. *African Journal of Basic & Applied Sciences*. **8** (*1*): 41-54.

Basiru,A., Ibukun,E.,Edobor,G and Onikanni, S.(2013). Qualitative And Quantitative Analysis Of Phytochemicals In Senecio Biafrae Leaf *International Journal Invention in Pharmaceutical Sciences*.,1(5);428-432.

Bergmeyer (1977) Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. IFCC method for aspartate aminotransferase. *Clinical Chemmistry*, 23: 887-899.

Dufour, D.R., Lott, J..A, Nolte, F.S., Gretch, D.R., Koff, R.S and Seeff, L.B.(2000). Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. *Clin Chemistry*,46 (12):2027-49.

Giannini,E. G., Testa,R and Savarino,V (2005). Liver enzyme alteration: a guide for clinicians. *Canadian Medical Association Journal*; 172(3): 367–379.

Gupta, M., Mazumder, U.K., Kumar, R.S., Sivakumar, T. & Vamsi, M.L. (2004). Antitumor activity and antioxidant status of *Caesalpinia bonducella* against Ehrlich ascetic carcinoma in Swiss albino mice. *Journal of Pharmacological Sciences.* **94** (*2*): 177-184.

Harborne, J.B. (1973). A guide to modern techniques of plant analysis. *In*: *Phytochemical methods*, Chapman and Hall Limited, London: 49-188.

Hye W. L., Hyojun K., Jin A. R. and Ki-yung K. (2014). Hemopoietic effect of extracts from constituent herbal medicine of samul-tang of phenylhydrazine-induced hemolytic anemia in rats. *International Journal of Clinical and Experimental Pathology*, **7**(9): 6179-6185.

Li J., Xia Y. and Kuter D. J. (1999). Interaction of thrombopoietin with the platelet complements receptor in plasma: binding, internalization, stability and pharmacokinetics. *British Journal of Haematology*, 106: 345-356.

Silitonga,M and Silitonga,P.M **(**2017**).** Haematological profile of rats (Rattus norvegicus) induced BCG and provided leaf extract of Plectranthus amboinicus Lour Spreng) Cite as: *AIP Conference Proceedings 1868, 090008* (2017); *https://doi.org/10.1063/1.4995200.* Assessed on 28/09/2020.

Sanghani,N.S ., Soundararajan, R. and Golper, T.A **(**2020). Serum enzymes in patients with kidney failure *https://www.uptodate.com/contents/serum- enzymes-in-patients- with-kidney-failure#H263399866.* Assessed 29/09/2020.

Ogbe, R. J., Adoga, G. I. and, Abu, A. H.(2010). Antianemic potentials of some plant extracts on phenyl hydrazine-induced anaemia in rabbits. *Journal of Medicinal plants Research*, **4**(8): 680-684.

Ohshima, H., Sawa, T and Akaike, T.(2006). 8-nitroguanine, a product of nitrative DNA damage caused by reactive nitrogen species: formation, occurrence, and implications in inflammation and carcinogenesis. *Antioxidant Redox Signal*; 8(5-6):1033-45.

Okokon,J.E., Antia, B.S., Udoh, A.E. and Akpan, M.M. (2007). Antianaemic and Antimicrobial Activity of *Eremomastax speciose*. *Journal of Pharmacology and Toxicology*;2 (2):196-199.

Okorie, U.C., Onu, J.A.,Ogunwa, S.C., Otuchristian , G., Njemanze, C.C., Ezeanya, C.F. and Igwe, D.O. (2021). Hematopoietic, Electrolyte and Glycemic Activities of *Whitfieldia lateritia* Leaf Decoction and Vitali Supplement on Phenylhydrazine-induced Anemic Cockerels. *Asian journal of Diary and Food Research;* 40 (3): 231-238.

Okorie,U.C.,Obasi,N.A.,Aja,P.M., Oje,O.,Ogwulumba,S.L. and Njoku, O.U. (2020a) Vitamins, Amino Acids and Antinutrient Compositions of *Whitfieldia Lateritia* Leaves. *Journal of Chemical, Biological and Physical Sciences*; 10 (2): 192-202.

Okorie,U.,Ogwulumba,S.,Otu,C.G.,Orinya,O.,Aniechi,S and Njoku,O.(2020b).Gas Chromatography -Mass Spectrometry Characterization of *W. lateritia* Leaves Decoction. *International Journal of Scientific & Engineering Research,* 11(8): 1560

Okorie, U.,Onu, J.A., Otu ,C.hristan, G., Otu Rachael Chioma, (2020c). Hepatotoxic Restorative Potential of *Whitfieldia Lateritia* Leaf Decoction and Vitali Supplement on Phenylhydrazine-Induced Anemic Cockerels. Global Journal of Science Frontier Research**,** *https://journalofscience.org/index.php/GJSFR/article/view/2772* Accessed: 15 may, 2022.

Singh, R. K., Pandey,K., Meena,A.K. and Akansha Jain (2014). Molecular Mechanism of Phenylhydrazine Induced Haematotoxicity: A Review. *American Journal of Phytomedicine and Clinical Therapeutics*; 2(3):390-394.

Soetan,K.O.,Akinrinde,A.. and Ajibade, T. O. (2013). *Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor).* Proceedings of 38th Annual Conference of Nigerian Society for Animal Production. Pp. 49-52.

Ali, S.A., .Sharief, N. H and Mohamed,Y.S.(2019).Hepatoprotective Activity of Some Medicinal Plants in Sudanhttps: Evidence-Based Complementary and Alternative Medicine//www.hindawi.com/journals/ecam/2019/2196315/**.** Assessed 25th September, 2020.

Toma, I.1., Victory, N. C.1 and Kabir, Y. (2015). The effect of aqueous leaf extract of fluted pumpkin on some hematological parameters and liver enzymes in 2,4-dinitrophenylhydrazine- induced anemic rats. African Journal of Biochemistry Research; 9(7): 95-98.

Vinoth S., Rajesh, K. P., Gurusaravanan, P and Jayabalan, N. (2011). Evaluation of phytochemical, antimicrobial and GC-MS analysis of extracts of *Indigofera trita L.F. spp*. Subulata (Vahl ex poir). *Intnational Journal of Agriultura.Reearch. 6(4):358-367.*

Wróblewski, F and LaDue, J.S. (1956). Serum glutamic-pyruvic transminase in cardiac and hepatic disease. *Proceedings of the Society for Experimental Biology and Medicine, 91: 569-571.write name of journal in full*

Radi,Z.A., KozaTaylor,P.H., Bell,R.R., Obert,L.A., Runnels,H.A., Beebe,J.S., Lawton,M.P and Sad is,S.(2011).Increased Serum Enzyme Levels Associated with Kupffer Cell Reduction with No Signs of Hepatic or Skeletal Muscle Injury. *American Journal Pathology*, 179(1): 240-247.