Hepatotoxic Restorative Potential of *Whitfieldia Lateritia* Leaf Decoction and Vitali Supplement on Phenylhydrazine-Induced Anemic Cockerels

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GJSFR-D Classification: FOR Code: 079999

HEPATOTOXICRESTORATIVEPOTENTIALOFWHITFIELDIALATERITIALEAFDECOCTIONANDVITALISUPPLEMENTONPHENYLHYDRAZINEINDUCEDANEMICCOCKERELS

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Results: Saponin, cardiac glycosides, tannins and flavonoids were found in the *W.lateritia* leaf decoction. The total feed intake was lower (p<0.05) in all the text groups except T10 (anaemic cockerels) treated with 400mg supplement which compared with the normal control. The feed conversion ratio was significantly decreased (p<0.05) in T10 when compared with the control and groups treated with decoction. There was a significant increase (P<0.05) in the weights gain and carcass weights of anaemic cockerels (T10) that received 400ng supplement compared to normal control and decoction test groups. The alanine amino transferase (ALT) activity of the test groups were significantly lower (P<0.05) when compared to the normal control, baseline control and negative control, except T4 that was treated with 400mg decoction only which was significantly higher (P<0.05) than the normal control. The AST activity of the test groups were significantly lower (P<0.05) when compared to the normal control, except T3 that was treated with 200mg decoction only which was significantly higher (P<0.05) than the normal control. The ALP activity of test groups were significantly higher (P>0.05) than the normal control, baseline control and negative control. Graded healing of the hepatocytes was observed among the different test groups with the anaemic groups (T8, T9, T10) treated with the vitali supplement showing the highest recovery.

Conclusion: The study showed that anaemic cockerels treated with vitali supplement had better hepatotoxic restoration that the anaemic cockerels treated with decoction.

Keywords: phytochemical, decoction, anaemia, feed conversion, whitfieldia lateritia.

I. Introduction

Plants have been a source of medicinal agents for thousands of year because of their nutritional and phytochemical compositions (Mithraja *et al.,* 2012). A remarkable number of modern drugs have been isolated from medicinal plants which have led to sudden increase in the number of herbal drug (Boopathi and Sivakumar, 2011). Medicinal plants with therapeutic properties are used for the treatment of many infectious diseases of humans as they contain many bioactive phytochemical constituents which are of curative effects (Sanaa *et al*., 2012). The medicinal properties of the plants are mainly due to the presence of secondary metabolites like alkaloids, cardiac glycosides, tannins, flavonoids, saponins, reducing compounds, minerals and vitamins However, the secondary metabolites are of great medicinal interest as they have significant biological activities and constitute the major actual active constituents of many crude drugs. Most of these constituents are not expressly safe as most of them can naturally be harmful or become toxic during in-vivo biotransformation, e.g. generation of reactive oxygen species. (Vinoth *et al*., 2011). Reactive oxygen species which create oxidative stress cause human diseases and disorders such as heart disease, inflammation, atherosclerosis, stroke, cancer, diabetes mellitus, (Rackova *et al*.,2007). The secondary metabolites possesses hepatoprotective and antioxidant effects through a scavenging action to the free radicals in the body by donation of electrons and avoiding the disruption of the cell membrane lipid bilayer structures, thus reducing the hepatotoxicity of the liver.). Due to the profitable efficacy of medicinal plants on biological activities, there is a profitable need to regularly investigate their biological activities with a view for

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possible isolation of newer biological compounds from plants that can serve as novel drugs (Gambhire *et al.*, 2009; Mirzaei *et al*., 2013). Plants that are under-studied and under-utilized are usually studied, and *W.lateritia* is one the plants used in folklore medicine but have been understudied. It is commonly called blood plant, and is a flowering plant belonging to the family of *acanthaceae* (D'Incalci *et al.*, 2005). *Whitfieldia lateritia* is a native plant in Sierra Leone but recently has been observed in several parts of the world like Nigeria. *Aja et al*., (2016) reported that *W.lateritia* contain alkaloids, flavonoid, saponins, tanins etc., and posses hypercholesterolemic property. *Whitfieldia lateritia* leaf is routinely used as cooking vegetable for the purpose of boosting haematological parameters by local people in various local governments' areas like Ikwo, Izzi, Afikpo South and Ivo in Ebonyi State of Nigeria yet no documentary evidence on the hepatotoxic effect (Okorie *et al.,* 2020; Aja *et al.,* 2015). This project was designed to investigate the hepatotoxic restorative potential of *W. lateritia* leaf decoction and vitali supplement on phenylhydrazine-induced anaemic cockerels

II.   Materials and Methods

a)   Collection of W. lateritia Plant Sample

Fresh leaves of *W. lateritia* plant were collected from izzi, Ebonyi state, Nigeria. The plant was identified by a taxonomist from the University of Port Harcourt. Some leaves were deposited their herbarium.

b)   Preparation of W.lateritia Leaf Decoction

The fresh leaves of *W.lateritia* were collected, destalked, weighed and washed under a running tap water. Twenty eight point six (28.6g) of the leaves was weighed with an analytical weighing balance, washed and then boiled with 300ml of distilled water at temperature of 70^0C for 15 minutes in order to extract the active ingredients of the plant in the form of decoction. Thereafter, the decoction extract was decanted into a clean container and stored in a refrigerator, and later used for the biochemical and histological studies..

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

c)   Phytochemical Screening

The phytochemical screening for the presence of tannins, saponins, alkaloids, cardiac glycoside, flavonoids and others were carried out on the aqueous leaf extract of *W.lateritia*.

❖   *Test for the presence of tannins*

This was carried out using Harbone method (1973).

Principle: Tannins are secondary metabolites of plant species and consist of sugar and non-sugar parts. They are capable of undergoing hydrolysis when inserted into dilute acids or boiling water to give rise to products such as poly hydroxyl phenolic compounds. They are reactive following the possession of functional groups called hydroxyl group (OH). They participate in redox reaction to give characteristics color change on the reagent applied.

Procedure: One milliliter (1ml) of crude extract of the sample was collected using syringe and dispensed into test tube. Then, one milliliter (1ml) of ferric chloride ($FeCl_3$) was added to the test tube. A dirty green precipitate was observed which showed the presence of tannins.

❖   *Test for the Presence of Saponins:* This was carried out by the method of Harborne (1973)

Principle: Saponins are glycosides with distinctive foaming characteristics. They consist of a polycyclic aglycone that is either a choline steroid or triterpeniod attached through C_3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called saponins. The ability of saponins to foam is caused by the combination of the non-polar sapogenin and the water soluble side chain (hydrophilic part), which have hydroxyl groups (-OH) as functional group.

Procedures:

Frothing Test: Two milliliters (2 mls) of the extract were diluted with 5 mls of distilled water in a test tube. The mixture was stirred vigorously for about 5mins and was allowed to stand for 30 minutes. Frothing which persisted for this duration indicated the presence of saponins.

❖   *Test For Presence of Alkaloids*

This was carried out by the method of Trease and Evans (1989)

Principle: Alkaloid can be detected as loose complexes following their ability to react with some reagents by producing characteristics color changes depending on the type of reagent used. Alkaloids have an amino group (NH_2) as their functional group as in nicotine.

Procedure: Two milliliters (2 mls) of the extract was collected using syringe and was dispensed into a test tube, the test tube was heated for 2 mins and 5mls of hydrogen (HCl) was added and heated again and allowed to cool. The mixture was divided into A and B. To A, 2 drops of Meyer's reagent was added and white precipitate was observed which showed the presence of Alkaloids. To B, 2 drops of Dragendroff's reagent was added and the formation of red precipitate was observed which confirmed the presence of alkaloids.

❖   *Test for the Presence of Flavonoids*

This was carried out by the method of Harborne (1973).

Principle: Flavonoids are colorless or pale yellow glycosides that are not soluble in non- polar solvents. They are compound that are oxidize by ethyl-acetate. They react with polar solvent to produce color changes

in accordance with the level of redox reactions that are likely to take place. Flavonoids also reacts with sodium hydroxyl group (NaOH) to form a yellow color following the reaction of the hydroxyl group (-OH) with the ketone functional group.

Procedure: Five milliliters (5 ml) of the extract was collected using syringe and was dispensed into a test tube. Exactly 10 mls of distilled water, 5mls of dilute ammonium hydroxide (NH_4OH) and few drops of tetraoxosulphate (VI) acid (H_2SO_4) were added in the test tube. A yellow coloration was observed which showed the presence of flavonoids.

❖ *Test for the Presence of Cardiac Glycoside*
This was carried out by the method of Harborne (1973)

Principles: Cardiac glycosides are organic compounds that are capable of undergoing hydrolysis in the presence of dilute acids, alkali or enzymes.

Procedure: Two milliliters (2 mls) of the extract was collected into a test tube and 5ml of glacial acetic acid was added and then 2mls of $FeCl_3$and 2mls of concentrated ferric acid were added too. A brown ring formation observed at inter phase of the mixture indicated the presence of deoxy sugar characteristics of cardiac glycosides.

d) *Design of Animal Experiment*
Sixty (60) cockerels were purchased and acclimatized for two weeks after which they were divided into 12 groups of 5 birds each, where Test groups T2 (100mg dc),T3(200mg dc) and T4(400mg dc) while T5 (100mg dc +PHZ),T6 (200mg dc +PHZ), and T7 (400mg dc +PHZ), T8 (100mg VS + PHZ),,T9(200mg VS + PHZ), and T10(400mg VS + PHZ and control groups T1(normal control),T11 (negative control) and T12 (baseline control).The birds were weighed and their initial weights recorded.
(VS: Vitali supplement, dc, decoction, PHZ phenylhydrazine)

e) *Induction of Anaemia*
Anaemia was induced through the injection of 10 mg/kg body weight of Phenylhydrazine for two consecutive days, after which the group T12 (baseline control). Blood was collected through ocular puncture; liver was collected into formalin and used for histological study.

f) *Clinical Signs*
Clinical signs or abnormalities observed upon induction of anaemia were: closing of the eyes, weakness, and loss of weight and itching of the eyes.

g) *Collection of Serum*
After 34 days of treatment, the final weights of the birds were taken before they were sacrificed. Whole blood sample were collected using syringe and centrifuged to get the serum for the biochemical analysis. The liver organ was carefully removed and preserved in formalin before taken to the laboratory for the histopathology analysis.

III. Biochemical Analysis

a) *Determination of Alanine Amino Transeferase Activity*
Alanine aminotransferase (ALT), formerly called serum glutamate-pyruvate transaminase (SGPT), catalyzes the transfer of amino group from alanine to keto-glutarate with the release of pyruvate and glutamate.

Principle: ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD. The rate of change of the absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD^+ and is directly proportional to the amount of ALT present in the sample (Wróblewski and La Due, 1956).

b) *Determination of Aspartate Amino Transeferase Activity*
Aspartate aminotransferase (AST), formerly known as glutamate-oxaloacetate transaminase (GOT) or serum glutamate–oxaloacetate transaminase (SGOT), catalyzes the transfer of the amino group from aspartate to α-ketoglutarate with the release of oxaloacetate and glutamate.

Principle: The aspartate aminotransferase (AST) test is based on the Karmen rate method (Karmen., 1955) as modified by Bergmeyer (Bergmeyer *et al*., 1977). AST catalyzes the reaction of L-aspartate and α-ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to NAD^+ by the enzyme malate dehydrogenase. The rate of absorbance change at 340 nm / 405 nm caused by the conversion of NADH to NAD^+ is directly proportional to the amount of AST present in the sample.

c) *Determination of Alkaline phosphatase Activity*
Principle: Alkaline phosphatase hydrolyzes *p*-NPP in a metal-ion buffer and forms *p*-nitrophenol and phosphate. The amount of ALP in the sample is proportional to the rate of increase in absorbance difference between 405 nm and 500 nm.

Procedure
The piccolo chemistry analyzer was turned on and allowed to completely boot.100 ul of the serum was collected using 100UL pipette. The pipetted serum was added into the piccolo cassette through a small dot on the cassette. The piccolo cassette was inserted into the piccolo chemistry analyzer and the result was displayed on the digital meter.

d) Histopathology Analysis on Tissue (Liver)

Histological method of processing tissue: this involves various ways of preparing, cutting, staining, and examination slides for histological report.

Stages in tissue processing:

(1) *Fixation:* The tissue was fixed in 10% formal saline in a container with light fitting lids for 3 day to prevent autolysis; improve staining quality and to aid optical differentiation of cell.

(2) *Dehydration:* The tissue was dehydrated to remove water that is not miscible with xylene and wax using different grades of alcohol ranging from 50% – absolute Alcohol for 30mins each

(3) *Clearing/De-alcoholization:* The dehydrated tissue was cleared by removing the alcohol from the tissue by immersing it through 3 changes of xylene for 30mins each.

(4) *Wax impregnation/ infiltration:* The cleared tissue was impregnated and infiltrated to remove the clearing agent (xylene) in the hot oven temp of 60^{0c} by passing it through three changes of molten paraffin was in a hot air oven for 30mins.

(5) *Embedding:* The infiltrated tissue was buried or embedded with molten paraffin wax in an embedded mould and allowed to solidify.

(6) *Mounting on wooding block:* The paraffin block of tissue was attached to a wooding block with the aid of a hot spatula held in between wood block and paraffin way, the spatula Melts the wax which solidifies when spatula was removed.

(7) *Microtomy:* The block of tissues was sectioned using rotary microtome, it was trimmed to obtain the cutting surface of the tissue at 15 micron and was sectioned at 5micron, and dry in hot plate for staining.

IV. STATISTICAL ANALYSIS

Data were analyzed using the statistical package for social science (Version 17). Difference among groups were measured using one way analysis of variance (ANOVA) followed by Duncan test. The results were expressed as mean ± standard deviation and differences were considered statistically significant at $P<0.05$.

V. RESULTS

Table 1: Phytochemical constituents of *W.lateritia* leaf decoction

S/N	Phytochemicals	Remarks
1	Alkaloids	-
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Cardiac glycosides	+

Keys: + = Present, - = Absent

Table 1 showed that the *Whitfieldia lateritia* leaf decoction contained appreciable amount of the following phytochemicals: flavonoids, saponins, tannins and cardiac glycosides. Our results (table 1) revealed the presence of bioactive compounds such as alkaloid flavonoids, saponins, tannins and cardiac glycosides. This observation is in line with the work of Aja *et al.,* (2016) who had observed similar phytochemicals in ethanol extract of *Whitfieldia lateritia* leaf. Previous reports had shown that these bioactive compounds are biochemically active in many respects; eliciting different arrays of biochemical activities (enzyme activation/ inactivation, antioxidant activities etc.). Flavonoids have been reported to possess antibacterial property as they have the capability to associate with soluble proteins and bacterial cell walls (Doss *et al.,* 2011). They also have antioxidant property as they inhibit oxidative stress by mitigation of the generation of free radicals, exhibit anti-inflammatory and anti-cancerous activity (Liu *et al*., 2008; Alsabri *et al.,* 2013). Flavonoids perform major roles in plants such as, protection against ultraviolet radiation, defense against pathogens and pests, pollen fertility, auxin transport regulation and pigmentation (Winkel, 2001). Similarly, saponins are reported to have antimicrobial (Rohit *et al.,* 2012) and anti-cancerous properties (Shi *et al.,* 2004). Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells (Rohit *et al.,* 2012). Tannins have antimicrobial, antidiarrheal, anti-inflammatory and antioxidant activities (Killedar and More, 2010). Cardiac glycosides are used for the treatment of cardiac failure as they can stimulate cardiac output by increasing the force of contraction as a result of their ability to increase intracellular calcium concentrations through binding to the extracellular surface of the ion transport protein in the cell, the membrane-inserted sodium potassium pump (Na$^+$/K$^+$-ATPase) (Xie and Askari, 2002).
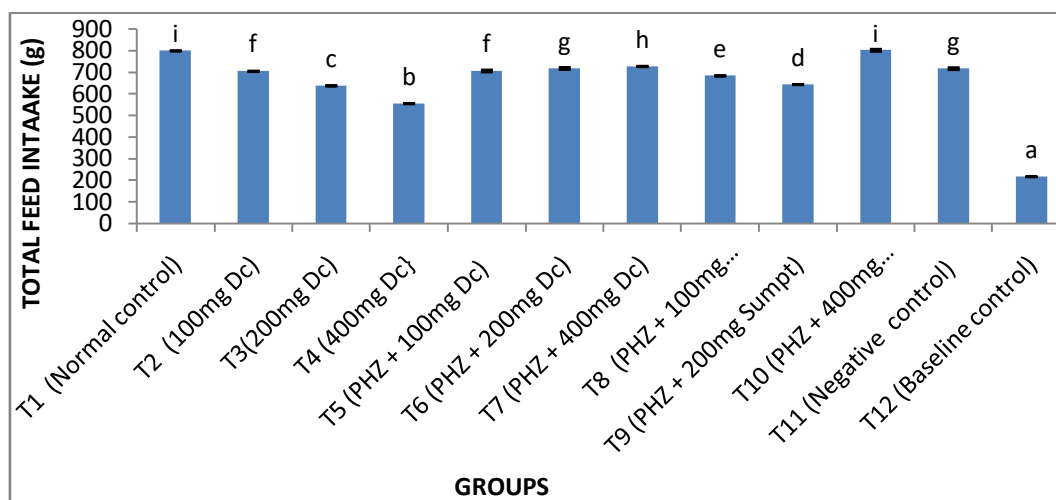
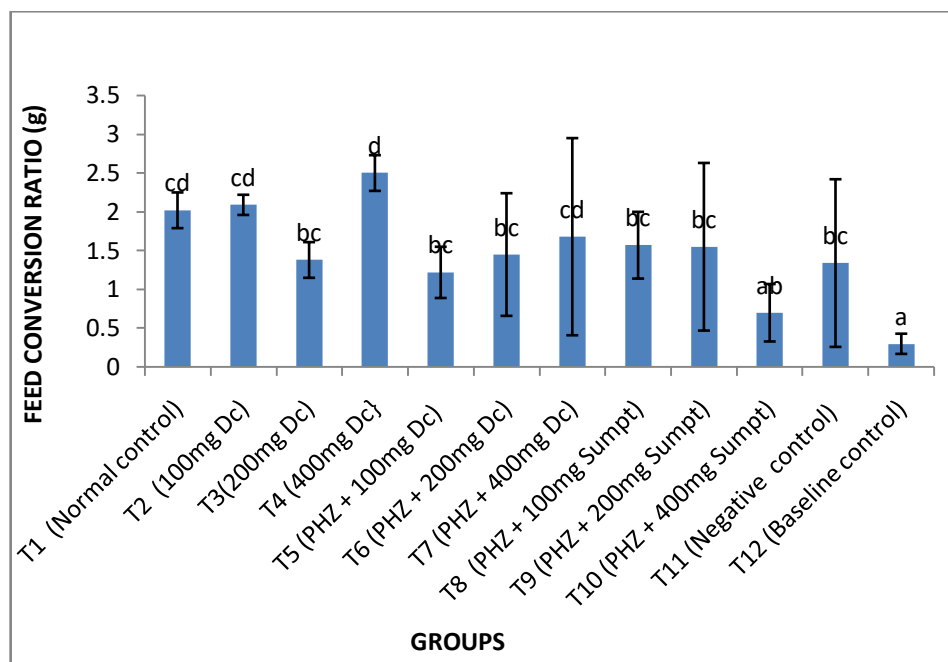*Mean ± SD, bars with similar superscript are not significantly different (P>0.05) n=5*

Fig. 1: Variations in the total feed intake of cockerels treated with *w. Lateritia* leaf decoction and vitali supplement

The total feed intake of the control was higher (p<0.05) than all the groups except group ten which was anaemic but treated with 400mg supplement. This observation agreed with the work of Machebe *et al.,* (2010) who reported a higher feed intake in birds fed vitalyte supplement than the extract of other leafy vegetables. This suggests that the vitali supplement might have stimulated appetite on the birds or increased their feed utilization leading to increased feed intake. The decreased feed intake witnessed in the decoction is consistent with the report of Chung *et al* (2010) who described decreased feed intake in birds treated with plant extracts due to possible effect of the bioactive components, since some phytochemicals such tannins had previously been reported to alter, negatively, feed intake in animal models. It can equally be inferred that the induction of anemia with phenylhydrazine with the attendant stressors, imposed a challenge on the metabolic capacity of the bird resulting to decrease feed intake, as this inferement had been corroborated by (Peter and Abel, 2006) who imputed that elevation of stress hormones will cause the body reserves to be mobilized to fuel the fight or flight response. Also nutrient absorption and gut motility decreases substantially during the stress response leading a decrease in feed intake.
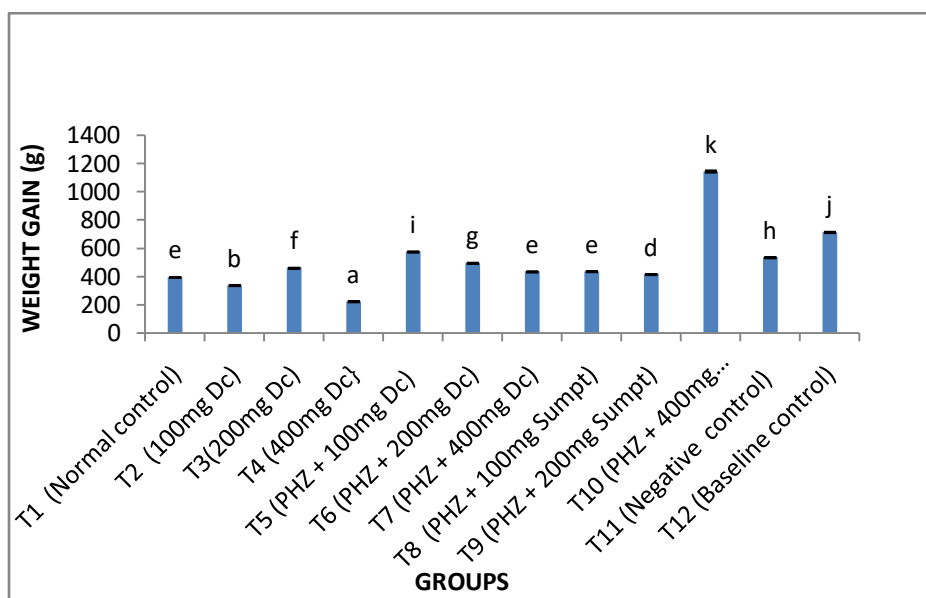

Mean ± SD, bars with similar superscript are not significantly different (P>0.05)

Fig 2: Variations in the Feed conversion ratio of cockerel treated with *W.lateritia* decoction leaf and V itali Supplement

The feed conversion ratio (figure 2) of the group 4, non anaemic group treated with the decoction only was significantly ($p<0.05$) increased when compared with the control and the group treated with the supplement This finding agrees with the work of Nworgu (2007) which showed that administration of leaf extract of fluted pumpkin can stimulate feed conversion ratio in cockerel. Feed conversion ratio measures the efficiency with which the bodies of live stock convert animal feed into the desired output. Feed conversion ratio is a function of the animal's genetics, age, the quality and ingredients of the feed. The cockerels in T10 had the highest feed efficiency while the other groups, especially those treated with the decoction exhibited low feed efficiency. This could be accounted for, by the possible negative interference of the bioactive components of the decoction on certain metabolic pathways by metabolic stimulating hormones, e.g. cathecholamines. Animals that have a low feed conversion ratio are considered efficient users of feed (Arthur *et al*., 2014).

Mean ± SD, bars with similar superscripts are not significantly different (P>0.05) n=5

Fig. 3: Variations in weight gain of anaemic cockerels treated with *W. lateritia* leaf decoction and vitali supplement

There was a significant increase ($P<0.05$) in the weights of anaemic cockerels (T10) that received 400ng supplement compared to normal control and other test groups (fig.3). The increased weight gains in conformity with earlier reports of Machebe *et al.,* (2011) who observed an increase in weight gain of birds fed vitalyte supplements. The increase in weight gain observed in group T10 can be attributed to the ability of VS to excite the olfactory nerves and taste buds, leading to an increase in feed consumption with the consequent increase in weight gain. An increase in the digestive secretion is one of the most important effects in improving the nutritional status (Platel and srinivisan, 2011). The actions that can produce these effects are increased salivary gland secretion, stimulation of mucus secretion in the stomach and intestine. Also weight gain can be as a result of increased secretion of pancreatic enzymes which accelerate digestion and shorten the time of feed passage through the digestive tract, improved digestion and absorption of lipids (Costa *et al.,* 2018). Furthermore, decreased weight gain noted in group 4 which was treated with decoction could be as a result of impaired feed intake and utilization as well as possible alteration of hormones responsible for animal growth and development.

Table 3: Variations in relative weight of the liver of cockerels treated with *W. lateritia* leaf decoction and vitali supplement

Groups	Relative Liver Weight (g)
T1 (Normal Control)	0.05 ± 0.02^a
T2 (100mg Dc)	0.05 ± 0.02^a
T3 (200mg Dc)	0.04 ± 0.02^a
T4 (400mg Dc)	0.05 ± 0.02^a
T5 (PHZ+100mg Dc)	0.04 ± 0.02^a
T6 (PHZ+200mg Dc)	0.05 ± 0.02^a
T7 (PHZ+400mg Dc)	0.05 ± 0.02^a
T8 (PHZ+100mg Supmt)	0.05 ± 0.02^a
T9 (PHZ+200mg Supmt)	0.05 ± 0.02^a
T10 (PHZ+400mg Supmt)	0.02 ± 0.02^b
T11 (Negative Control)	0.04 ± 0.02^a
T12 (Baseline Control)	0.03 ± 0.02^b

Mean ± SD, values with similar superscripts are not significantly different (P>0.05) n=5

The relative weight of the liver of the test groups were not significantly different (p>0.05) from that of the normal control. The groups that were PHZ-induced and treated with decoction were not significantly (p>0.05) different from those that were induced and treated with vitali supplement (p>0.05) except for T10. Increase in the relative weight of animal liver can occur when there is accumulation of tiacyglycerol leading to enlarged liver consequent upon increased influx of fatty acids into the liver or de novo synthesis of the fatty acids in liver and or low capacity of excretion of lipoprotein from the liver resulting from a deficiency of apolipoprotein B synthesis, and ecreased VLDL is one of the causes of lipid accumulation in the liver; this occurred when the protein synthesizing materials (e. g. choline) are in short supply (Ohno *et al.,* 2000).
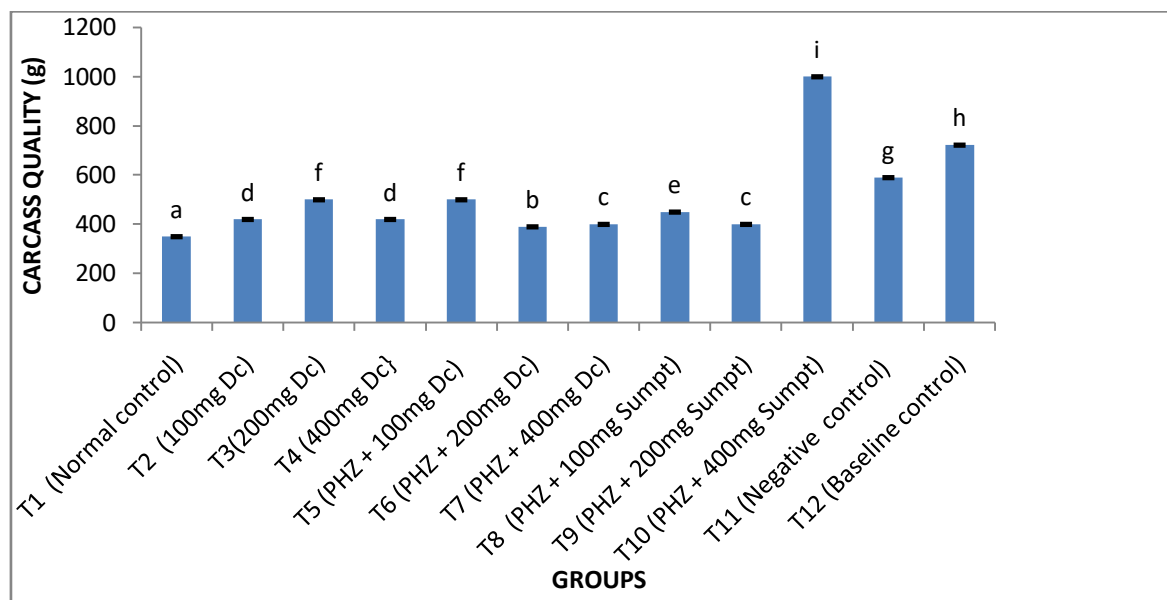
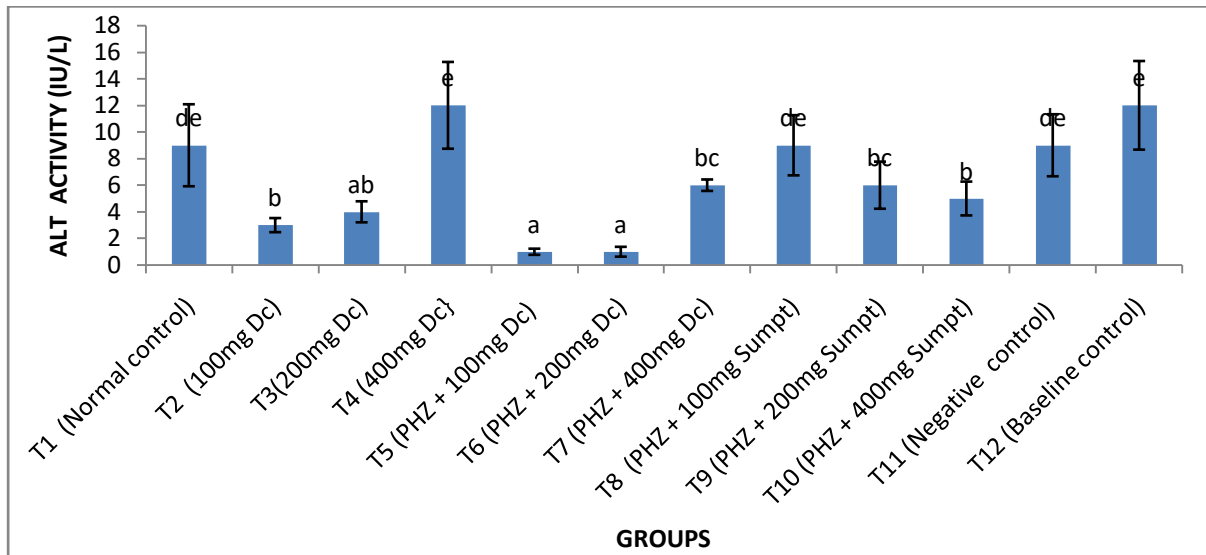*Mean ± SD, bars with similar superscripts are not significantly different (P>0.05) n=5*

Fig. 4: Variations in carcass weight of cockerels treated with *W. lateritia* leaf decoction and Vitali supplement

There was a significant increase (P<0.05) in the carcass weight of test group, T10, compared to normal control and other test groups (figure 4). The elevated carcass weight observed in this study was not in conformity with the earlier reports of Esonu *et al.,* (2006) who observed that the administration of neem extract in poultry stimulated carcass weight increase than the group that received supplement. Carcass weight can be affected by dietary protein content of animal feed. A diet with lower protein reduces the yield of meat and increases the fat content of the carcass, also high bird density may induce competition for space, lower feed intake, leading to poor feathering, skin lesions and decreases the carcass quality. Dim lights have been observed to increase fat levels of carcass, as adequate light is responsible for the stimulation of receptors responsible for the release of growth hormones which act as lipolytic agents in the body, however, under dim

lights the concentrations of these hormones is decreased (Garcia *et al* ., 2002).

Mean ± SD, values with the similar superscript are not significantly different (P>0.05). n=5

Fig. 5: Variations in ALT activity of cockerels treated with *W. lateritia* leaf decoction and vitali supplement

The alanine amino transferase (ALT) activity of the test groups were significantly lower ($P<0.05$) when compared to the normal control, baseline control and negative control, except T4 that was treated with 400mg decoction only which was significantly higher ($P<0.05$) than the normal control. Our current findings (figure 4.5) revealed decreased ($p<0.05$) alanine amino transferase (ALT) activity of the test groups when compared to the normal control, baseline control and negative control.

The decrease in ALT activity observed in the test groups did not agree with the work of Duru *et al.,* (2018) who observed a significant increase ($p<0.05$) in ALT activity of rats fed the bark of *Carica papaya extract*, although there was an observed increase ($p<0.05$) in the test group, T4 which was treated with 400mg decoction only in our present study. The decreased level of ALT activity observed in the decoction treated groups compared with the control could be due to impairment of protein synthesis through various mechanisms such as interference at the gene expression or protein inactivation, of which tannin is a known culprit. The supplement treated grouped which did not differ ($p>0.05$) from the control could be because the supplements lacks the necessary ingredients required for growth and tissue repair. ALT is an enzyme that is mostly used in clinical biomarker for monitoring liver disease (Ozer *et al.,* 2008). ALT which is responsible for the metabolism of alanine is found in much higher concentration in the liver compared to other organs. The observed increase in ALT activity of T4 and baseline control can be an indication of liver damage (Edgar *et al.,* 1998). When hepatocellular injury such as necrosis or membrane damage occurs, the liver abundant

enzyme ALT will leak into the extracellular space and enter the blood where it shows a slow clearance rate (Ozer *et al.,* 2008). However, despite the specificity of ALT levels to hepatocellular disease, the absolute peak of the ALT elevations does not correlate with the extent of liver cell damage (Mathiesen *et al.,* 1999). Extrahepatic injury such as muscle injury can lead to an elevation in ALT thereby making ALT not entirely hepatospecific, also fenofibrinate has been found to increase ALT gene expression in the absence of apparent liver injury (false positive), (Edgar *et al.,* 1998).
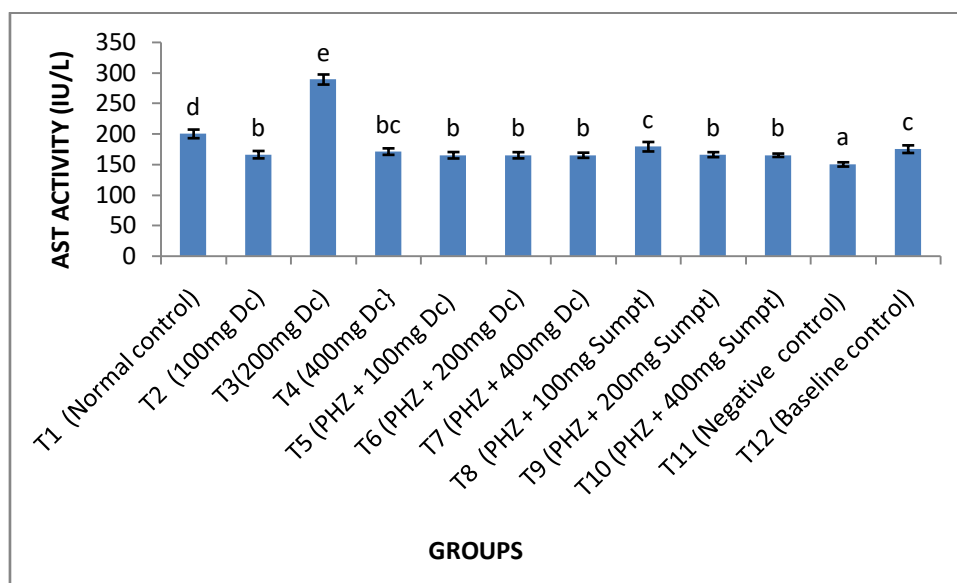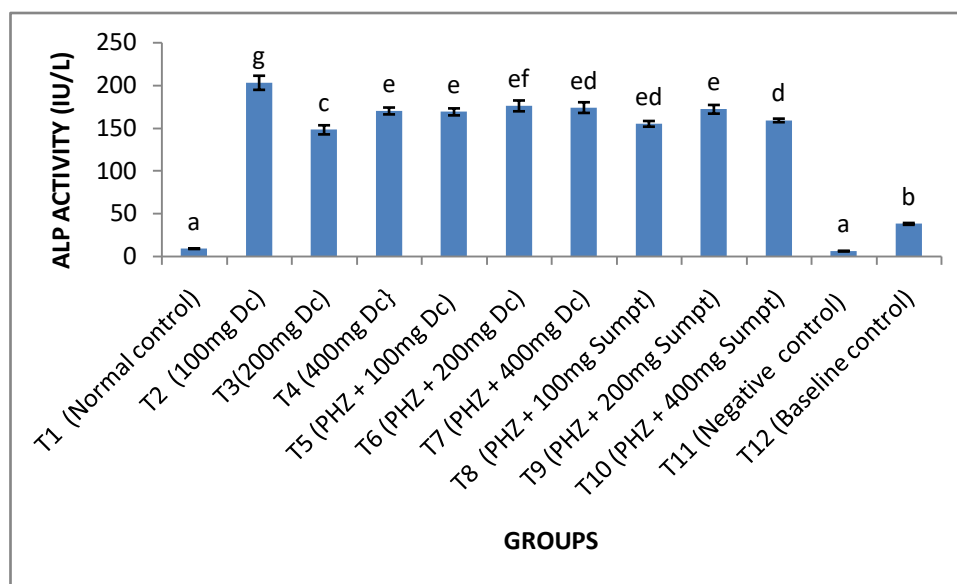
Mean ± SD, bars with the similar superscript are not significantly different (P>0.05). n=5

Fig. 6: Variations in AST activity of cockerels treated with *W. lateritia* leaf decoction and vitali supplement

The AST activity of the test groups were significantly lower (P<0.05) when compared to the normal control, except T3 that was treated with 200mg decoction only which was significantly higher (P<0.05) than the normal control (Figure 6).

In the present study, AST activity (figure 6) was decreased (p<0.05) in the test group when compared with the control. The decreased AST activity reported in the test group relative to the control was not in conformity with the work of Duru *et al.,* (2012) who observed a significant increase (P<0.05) in AST activity of rats fed the bark of *Carica papaya crude* extract relative to their control. The discrepancies in both studies could be as a result of the different treatments of the animals in both studies such as induction anemia, as in the case of our study, different dosages used and duration of studies. AST which is less specific than ALT, is found in highest amount in extra hepatic organs, such as heart and muscle. Increased AST activity in the test group T3 can be as a result of cellular leakage and loss of functional integrity of the cell membrane of the hepatocytes. Of course, this is understandable since alteration of membrane integrity through lipid peroxidation of membrane lipid is a typical toxic effect of phenylhydrazine which was used to induce anemia. This also conformed to the histological analysis that revealed a recovery from hepatic inflammation of the birds. However, diseases such as viral hepatitis, necrosis, cardiac infarction and muscle injury can equally elicit the elevation of AST (Robert *et al.,* 2017, 2000).

Mean ± SD, bars with the similar superscript are not significantly different (P>0.05). n=5

*Fig. 7:* Variations in ALP activity of cockerels treated with *W. lateritia* leaf decoction and vitali supplement

The ALP activity of test groups were significantly higher (P>0.05) than the normal control, baseline control and negative control (figure 7). But the test groups that were PHZ-induced and treated with decoction were not significantly different (P>0.05) from the test groups that were PHZ-induced and treated with vitali supplement except for T2 which showed highest (P<0.05) ALP activity. In the present study, ALP activity of the test groups agreed was observed to increase when compared with normal control, this is in conformity with the work of Andongma, (2014) who had reported an increase in ALP activity in PHZ-induced anaemic rats that received methanolic extract of *Gnetum africanum* leaves. The increased ALP activity relative to the normal control could be due to the stimulation of protein synthesis cum disruption of cellular membrane of the hepatoctyes, small intestine, and bone. Also, elevated ALP activity seen in the test groups could be as a result of blockage of bile ducts (Robert *et al*.,2017 and Mathew *et al.,* 2000). This observed elevation in ALP is in tandem with the histopatholgy result that revealed hepatoxicity. ALP is found in the kidney, bone, liver and placenta, but its activity is mainly in the liver. Infiltrative liver diseases such as abscesses, granulomatous and amyloidosis may also cause an increase in ALP activity (Fuchs *et al.,* 2008). While the decreased level in the negative control observed in the current study might be attributed to ALP synthesis impairment, zinc deficiency, vitamin B12 deficiency since availability of both nutrients can be compromised in an anaemic condition. Other conditions that could lead to low levels of serum ALP include hypothyroidism and congenital hypophosphatasia which could explain the low ALP activity since phenylhuydrazine and its the metabolic products could act through different mechanism to warrant compromise of materials needed for the development of the bone and thyroid gland (Agbai *et al* ., 2014 and Clermont and Chalmers, 1967).
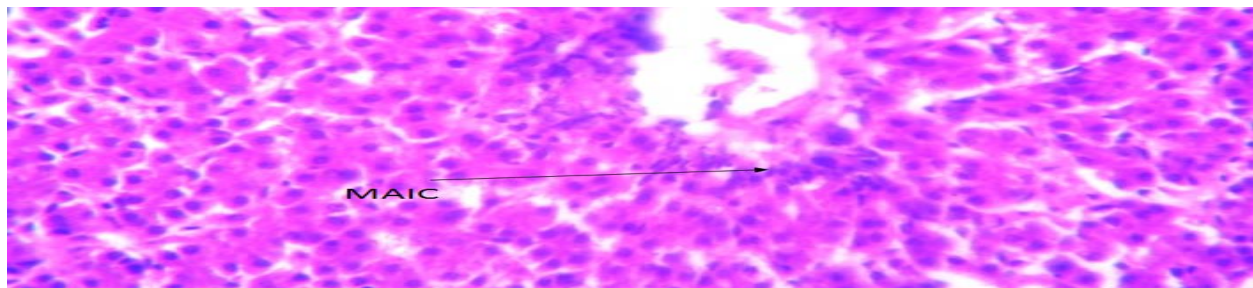
Histopathology
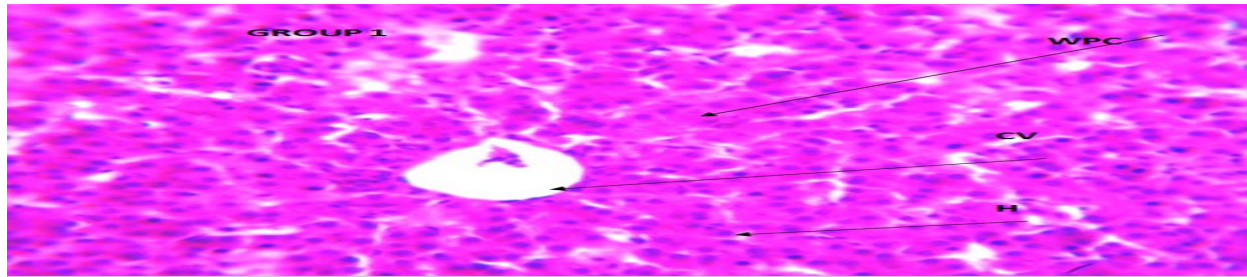
Fig. 8: Group T1: normal control received distilled, water section of liver (x400)(H/E) shows normal well perfused hepatic architecture with central vain (C),cytoplasm (C) and hepatocyte (H).
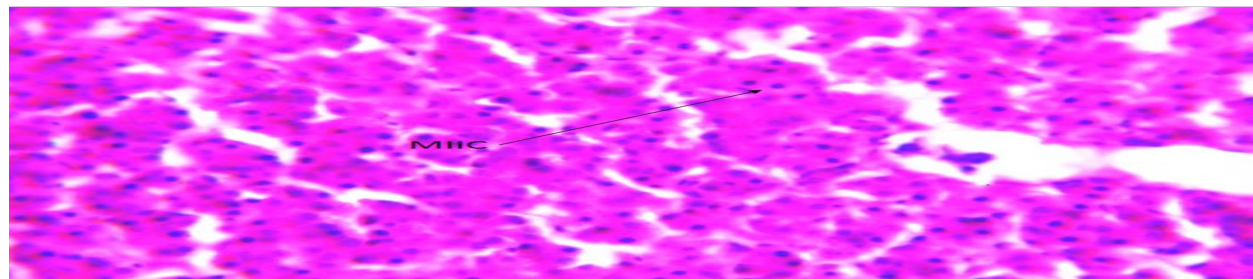
Fig. 9: Group T2 100mg decoction only, section of liver (x400) (H/E)shows hepatic tissue with mild infilteration of inflammatory cell (MIIC) otherwise normal
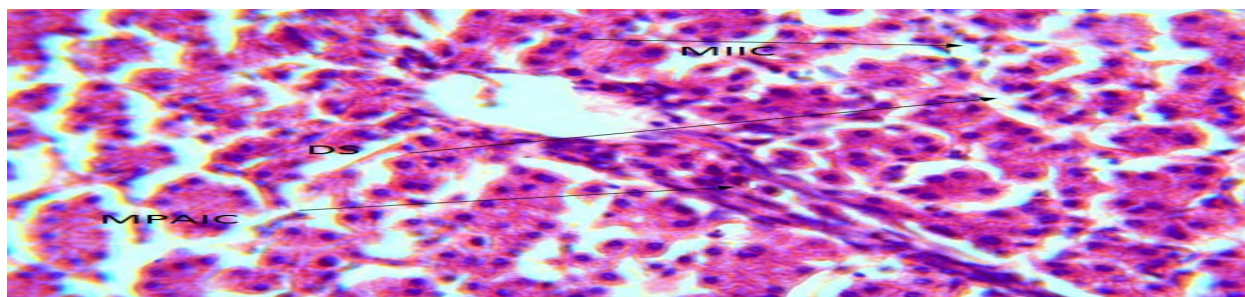

Fig. 10: Group T3:200mg decoction only section of liver (x60,150) (H/E) shows hepatic tissue with mild focal aggregate inflammatory cell (FAIC) around the central vain otherwise normal
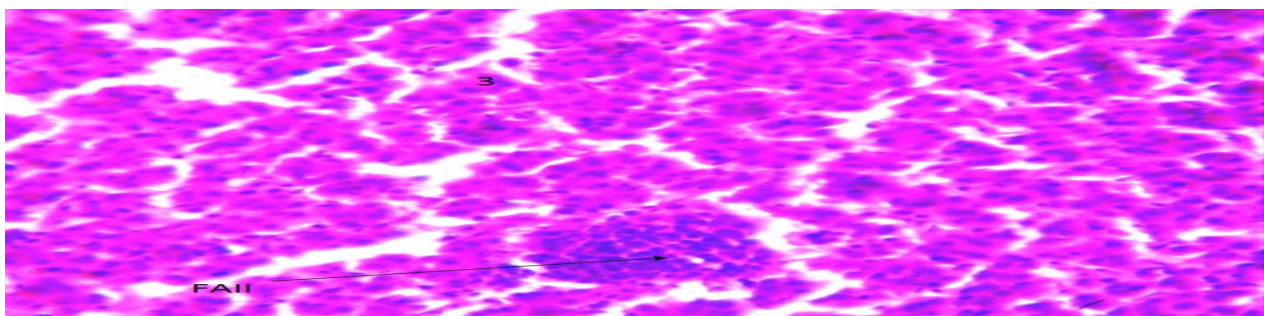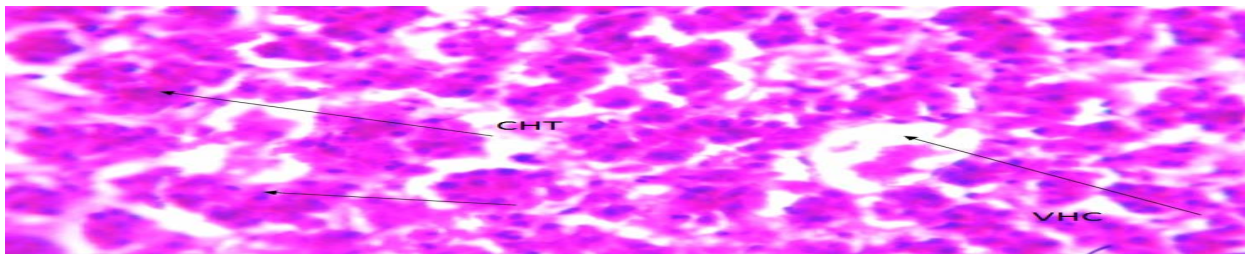

Fig. 11: Group T4: decoction only of section of liver (x400) (H/E) shows hepatic tissue with moderate focal aggregate of intrahepaticinflammatory cell (FAIIC)
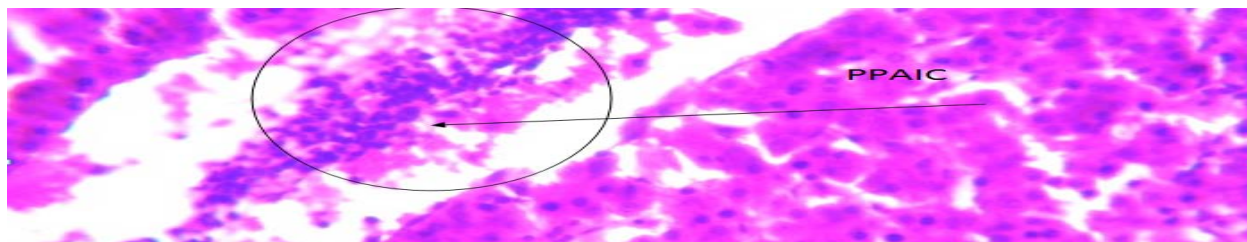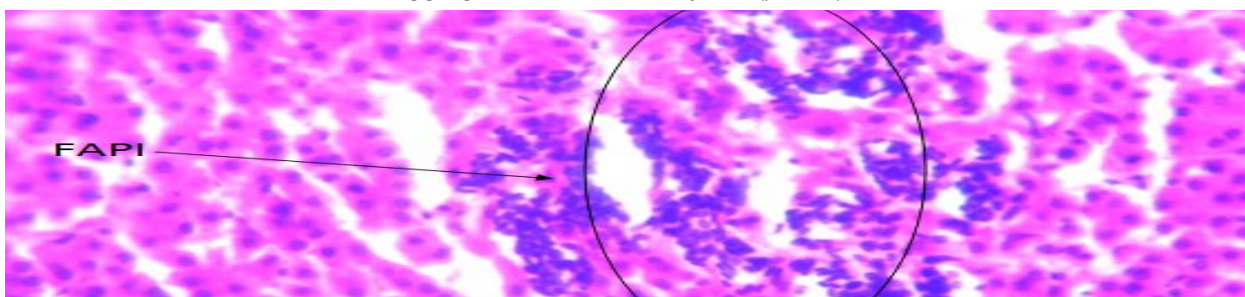
Fig. 12: Group T5: PHZ + 100mg decoction, section of liver (x400) (H/E) shows hepatic tissue with moderate portal aggregate of inflammatory cell (MPAIC), dilation of the senosoid (DS) and clumping of the hepatic tissue (CHT)


Fig.13: Group T6: PHZ + 200mg decoction, section of liver (x400) (H/E) shows hepatic tissue with clumping (C) moderate infinfilteration of inflammatory cell (MIIC) and vacoulation hepatic tissue(VHT)

Fig.14: Group T7: PHZ + 400mg decoction, section of liver (x400) (H/E)shows hepatic tissue with peri portal aggregate of inflammatory cell (pPAIC) .
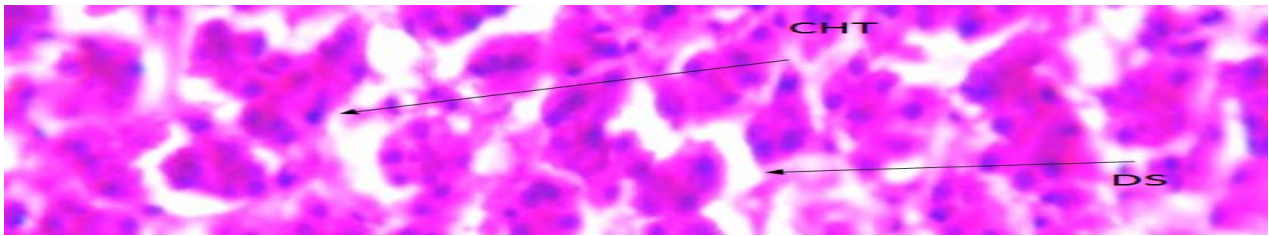

Fig. 15: Group T8: PHZ+100mg vitali supplement, section of liver (x400) (H/E)shows hepatic tissue with moderate to sever peri portal aggregate of inflammatory cell(PPAI)
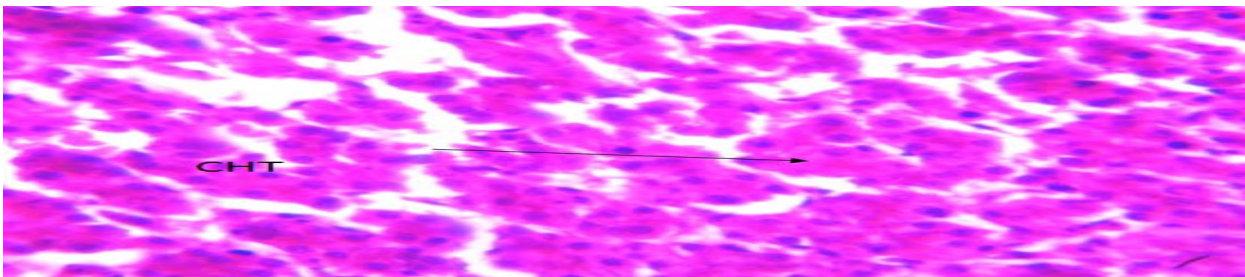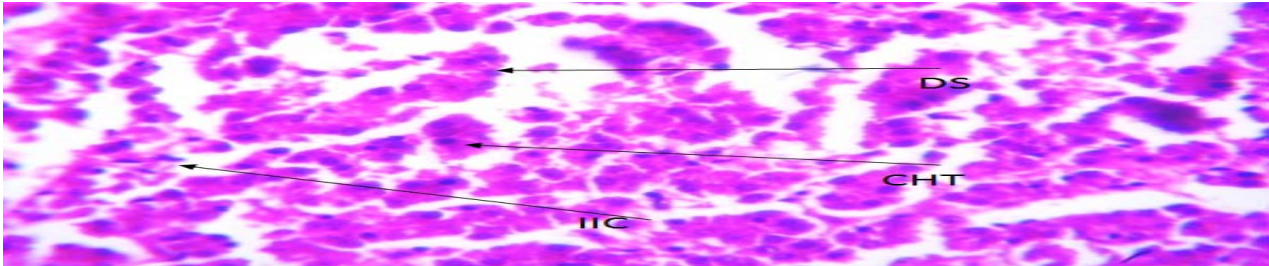

Fig.16: Group T9: PHZ+200mg vitali supplement, section of liver (x400) (H/E)shows hepatic tissue with mild clumping of the hepatic tissue(CHT) otherwise normal

Fig.17: Group T10: PHZ+400mg vitali supplement, section of liver (x400) (H/E)shows hepatic tissue with moderate dilation of the senosoid (DS) and clumping of the hepatic tissue (CHT)

Fig.18: Group T11: PHZ(anaemic, not treatment), negative control; section of liver (x400) (H/E) shows disorganised hepatic tissue with moderate to severe ,dilation of the senosoid (DS), clumping of the hepatic tissue (CHT) and infilteration of inflammatory cell(IIC)
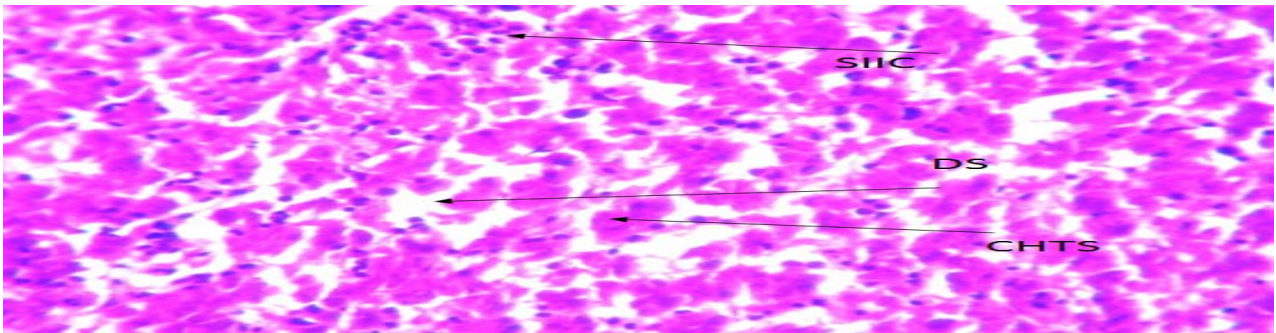
Fig. 19: Group T12: PHZ, baseline control, section of liver (x400) (H/E) shows severe disintegration of hepatic architecture with dilation of the sinusoid (DS), clumping of the hepatic tissue (CHT) and severe infiltration of inflammatory cell(SIIC)

The histopathological results (Figures 8-19) revealed compromised liver integrity in the baseline control (fig 19), negative control (fig 19) as against normal hepatic architecture in the normal control-T1 (fig 8). But in the test groups, graded healing of the hepatocytes was observed among the different test groups with the groups (T8, T9 and T10) that were PHZ-induced but treated with the supplement showing the highest recovery. The histological finding had indicated that the supplement treated cockerels have better hepatotoxic healing potential than the *W. lateritia* leave decoction treated groups.

VI. CONCLUSION

The results from this study revealed that anaemic cockerels treated with vitali supplement showed better performance, which are evident in the increased weight gain, carcass weight, lower activity of serum liver enzymes and observed liver injury recovery when compared with test groups that were treated with the *W. lateritia* leaf decoction. Groups that were treated with lower dosage of *W. lateritia* leaf decoction showed a better performance than those that were treated with higher doses of the decoction. Finally, vitali supplement have a better protective effect on the cockerels than *W. lateritia* leaf decoction and can be used in improving the general well being of cockerels.

REFERENCES RÉFÉRENCES REFERENCIAS

1. Agbai Emmanuel Onuka, Mounmbegna Pouonkoussou Emmanuel and Nwafor Arthur (2014). The

Beneficial Effects of Methanol Extract of Annona Muricata Seeds on Acute Hemolytic Anemia in Albino Wistar Rats. *Journal of Medical Science And Clinical Research*; 2(5):958-964.

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

3. Aja, P.M., Okorie, U. C., Ozougwu, V. E. O., Onye-Mmaghiri, E. A., Agu, K. A., Nweke. O. I. (2016). Phytochemical and Gas Chromatography-Mass Spectrometric (GC- MS) Analyses of *Whitfieldia lateritia* Leaf, *African journal of Basic & applied science, 41-54.*

4. Alsabri SG, El-Basir HM, Rmeli NB, Mohamed SB, Allafi AA, Zetrini AA, Salem AA, Mohamed SS, Gbaj A, El-Baseir (2013). Phytochemical screening, antioxidant, antimicrobial and anti-proliferative activities study of Arbutus pavarii plant. *J. Chem. Pharm. Res*. 5(1):32-36.

5. Arthur, P.F., Jennie E. Pryce and Robert M Herd (2014). Lessons Learnt from 25 Years of Feed Efficiency Research in Australia. Proceedings, 10th World Congress of Genetics Applied to Livestock Production.

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

7. Boopathi A. C., Sivakumar R. (2011). Phytochemical screening studies on the leaves and stem of *Andrographis neesiana wight* - An endemic medicinal plant from India. *World App. Sci. J. 12(3):307-311*

8. Chung, T.K., Khajarern. J. and Khajarern S. Effect of dietary threonine on growth performance and carcass characteristics of broiler chickens raised at high temperature. Singapore: Department of Animal Sciences, Faculty of Agriculture, Khan Keen University; 1995.

9. Clermont RJ, Chalmers TC (1967). The transaminase tests in liver disease. *Medicine;* 1967: 46(2):197-207.

10. Costa, A. S. H., Costa, P., Alves, S. P., Alfaia, C. M., Prates, J. A. M., Vleck, V., ... Bessa, R. J. B. (2018). Does growth path influence beef lipid deposition and fatty acid composition?. PLoS ONE, *13*(8), e0193875.

11. D'Incalci, M., Steward, W.P., and Escher, A.Y. (2005). Use of Cancer Chemo Preventive phytochemical as Anti-neoplastic Agents. *Lancetonocology, 6(67):899-904.*

12. Doss A, Parivuguna V, VijayaSanthi M, Sruthi S. 2011. Antibacterial and preliminary phytochemical analysis of Medicago sativa L. against some microbial pathogens. Indian J. Sci. Tech. 4(5):550-552.

13. Duru M, Amadike U, Amadi B (2013). Effect of Solanum macrocarpon fruit on haematology, hepatic and renal function. *Advances in Biochemistry* 1(2):28-32

14. Edgar, A.D., Tomkiewicz, C., Costet, P.(1998). Fenofibrate modifies transaminase gene expression via a peroxisome proliferator activated receptor alpha-dependent pathway. *Toxicol Lett 98: 13-23*.

15. Esonu, B.O., Oliver O Emenalom, Abi Udedibie , Madu, U. and Inyang AO (2006)Evaluation of Neem (Azadirachta indica) leaf meal on performance, carcass characteristics and egg quality of laying hens. International Journal of Agriculture and Rural Development 6(1).

16. Fuchs RK, Allen MR, Condon KW, Reinwald S, Miller LM, McClenathan D, Keck B, Phipps RJ, Burr DB (2008) Calculating clinically relevant drug doses to use in animal studies. *Osteoporos Int* 19:1815–1817.

17. Fujita H. (1939) Umber die Mikrobestimmung der Blutphosphatase. *J Biochem, Japan, 30: 69-87.*

18. Gambhire, M. N., Wankhede, S. S., Juvekar, A.R. (2009). Antiinflammatory activity of aqueous extract of Barleria cristata leaves. *Pharmacogn. 1:222-224.*

19. García, M.L. , R. Dominguez,R, Galvez, M.D. , Casas, C. , . Selgas, M.D (2002). Utilization of cereal and fruit fibres in low fat dry fermented sausages. *Meat Science*; 60(3):227-236

20. Harbone, J. B. C. (1973) Phytochemical methods. *Chapman and Hall, London P.279.*

21. Kalpana Platel and Krishnapura Srinivasan (2000) Influence of dietary spices or their active principles on pancreatic digestive enzymes in albino rats. *Food / Nahrung;* 44(1):42-6.

22. Killedar SG, More HN. 2010. Estimation of tannins in different parts of Memecylon umbellatum Burm. J. Pharm. Res. 3(3):554- 556.

23. King, E.J, Armstrong, A.R. (1931)A convenient method for determining serum and bile phosphatase activity. *Can Med Assoc J, 31: 376-381.*

24. Kumar, G., Sharmila, Banu G., Murugesan, A.G., Rajasekara, Pandian M. (2006). Hypoglycaemic effect of *Helicteres isorabark* extract in rats. *Journal Ethnopharmacology, 107:304-307*.

25. Liu X, Zhao M, Wang J, Yang B, Jiang Y. 2008. Antioxidant activity of methanolic extract of emblica fruit (Phyllanthus emblica L.) from six regions in China. J. Food Compos. Anal. 21(3):219-228.

26. Machebe NS, Agbo CU, Onuaguluchi CC. Performance of finisher broilers served Gongronema latifolia Benth. Leaf extracts as supplementary source of vitamins and minerals. In: Proceedings of the 15th Annual Conference of Animal Science Association of Nigeria; 2010.

27. Mathews SC, Narotsky DL, Bernholt DL, Vogt M, Hsieh Y-H, Pronovost PJ,(2012) Pharm JC. Mortality among marathon runners in the United States, 2000–2009. Am J Sports Med.; 40(7):1495–500.

28. Mathiesen, U.L. , Franzén, L.E. , Frydén, A. , Foberg, U. and Bodemar, G. (1999).The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. *Scand. J. Gastroenterol*., 34 (1) (1999): 85-91.

29. Mirzaei A, Toori., M. A, Mirzaei N., Shirazi R. (2013). Antioxidant, antimicrobial and antimutogenic potential of four Iranian medicinal plants. *Life Sci. J. 10(7):1085-1091*.

30. Mithraja, M. J., Irudayaraj D., Kiruba S., Jeeva S.(2012). Antibacterial efficacy of *Drynaria quercifolia* (L.) J. Smith (Polypodiaceae) against clinically isolated urinary tract pathogens. *Asian Pac. J. Trop. Biomed. S131-S135.*

31. Nkwocha Chinelo and Obioma U. Njoku (2015) Effect of methanol extract of Synsepalum dulcifium pulp on some biochemical parameters in albino rats. *Journal of Coastal Life Medicine* 3(3)

32. Nworgu FC. (2007). Economic importance and growth rate of broiler chickens served fluted pumpkin (*Telferia occidentalis*) leaves extract. *African Journal of Biotechnology*. 6(2):167–174.

33. Ohno,Y, S. Suto, M. Yamanaka, Y. Mizutani, S. Mitsu take, Y. Igarashi, T. Sassa, A. Kihara (2010) . ELOVL1 production of C24 acyl-CoAs is linked to C24 sphingolipid synthesis Proc. Natl. Acad. Sci. USA, 18439-18444 20937905 107 pp.

34. Okorie,U.C. Obasi,N.A. Aja P.M., Oje,O. ,Ogwulumba,S.L. and Njoku, O.U. (2020) Vitamins, Amino Acids and Antinutrient Compositions of *Whitfieldia Lateritia* Leaves. *Journal of Chemical, Biological and Physical Sciences*; 10, No. 2; 192-202,

35. Ozer, J., Ratner, M., Shaw, M., Bailey, W., and Schomaker, S. (2008) The current state of serum biomarkers of hepatotoxicity. *Toxicology 245: 194-205.*

36. Peter R. Ferket and Abel G. Gernat (2006)Factors That Affect Feed Intake of Meat Birds: A Review. *International Journal of Poultry Science*;5(10): 905-911

37. Petitclerc C (1975). Mechanism of action of Mg2+ and Zn2+ on rat placental alkaline phosphatase. I. Studies on the soluble Zn^{2+} and Mg^{2+} alkaline phosphatase. *Can Journal Biochemistry, 53: 1089-1100.*

38. Rackova L., Oblozinsky D., Kostalova V., Kettmann V., Bezakova L.(2007). Free radical scavenging activity and lipoxygenase inhibition of Mahonia aquifolium extract and isoquinoline alkaloids. *J. Inflamm. 4:15-22.*

39. Reitman S and Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol, 28: 56-63*.

40. Robert, C. Thomas R. Hustead, M.D., syed M. Ali, MD, Matthew w. Pantsari, MD, (2017).*am fam physician;* 1; 96(11): 709-715.

41. Rohit S, Thakur GS, Sanodiya BS, Mukeshwar P, Prakash B. 2012. Saponin: a wonder drug from Chlorophytum species. Global J. Res. Med. Plants Indigen. Med. 1(10):503-515.

42. Sanaa M., Shananab M., Emad, A.S. (2012). Biological activates and anticorrosion efficiency of water hyacinth (*Eichhornia crassipes). J. of Med. Pl. Res. 6(23):3950-3462.*

43. Shi, Q., Hong, H., Senior, J., and Tong, W. (2010) Biomarkers for druginduced liver injury. *Expert Rev Gastroenterol Hepatol 4: 225-234.*

44. Trease GE, Evans WC. 1987. Pharmacognosy. Bailliare Tindall, London.

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

46. Winkel-Shirley, B. (2001). Update on flavonoid biosynthesis: Flavonoid Biosynthesis. A Colorful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. *Plant Physiology.126 (2): 485-493.*

47. Wróblewski F, LaDue, J.S. (1956) Serum glutamic-pyruvic transminase in cardiac and hepatic disease. *Proc Soc Exp Biol Med, 91: 569-571.*

48. Xie, Z. and Askari, A. (2002). Na+/K+-ATPase as a signal tranducer. *European Journal of biochemistry 269(10): 2434-2439.*