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Hepato-protective potentials of aqueous, chloroform and methanol leaf extracts of *Whitfieldia lateritia* on 2, 4-dinitrophenylhydrazine-induced anaemia in rats

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Abstract

This study aimed at investigating the hepato-protective potentials of the aqueous, chloroform and methanol leaf extracts of *Whitfieldia lateritia* on 2, 4-dinitrophenylhydrazine (2,4-DNPH)-induced anaemia in rats. The toxicity study, quantitative phytochemical screening, total and direct bilirubin concentrations, mean protein, albumin and globulin concentrations, as well as mean liver marker enzymes activities (ALT, AST and ALP) were carried out using standard procedures. Thirty-six wistar rats were grouped into six (n =6). Group I: normal control; Group II: negative control; Group III: administered 0.6 ml/kg body weight (b.w) of Astifer (standard Haematinic); Group IV to VI were administered 400 mg/kg b. w. of the aqueous, chloroform and methanol leaf extracts, respectively. Induction of anaemia was achieved in the test groups (II-VI) by administration of 2, 4-dinitrophenylhydrazine (20 mg/kg b.w.) once daily for seven days. Administration of extracts commenced subsequently and lasted for 21 days. Animals were sacrificed on the 22nd day and blood collected for laboratory analysis. ALT, AST and ALP activities of group II anaemic rats showed significant ($P < 0.05$) reduction compared with normal control rats. Group III rats showed significant ($P < 0.05$) increase in ALT, AST and ALP activities compared with group II anaemic rats. Group IV rats showed significant ($P < 0.05$) increase in ALT and AST activity compared with group III rats. The total bilirubin concentration of group II rats was non-significantly ($P > 0.05$) higher compared with the normal control rats. Groups IV and VI rats showed non-significant ($P > 0.05$) reduction in total bilirubin concentration compared with group V rats. In conclusion, *W. lateritia* leaf has beneficial hepato-protective properties in Wistar rats at therapeutic dose that supports its use in the treatment of hepatic diseases.

Keywords: Hepato-protective, Plants, Anaemia, Extracts, *Whitfieldia lateritia*

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INTRODUCTION

Plants are primary sources of medicine, food, shelter, fiber and other necessities used every day by humans. The roots, stem, flowers, leaves, seeds and fruits provide food for animals and human beings (Edeoga*et al.,* 2005). The consumption of plants is not just for its nutrient value but also for its medicinal effects or purposes and even in modern times plants are being used in many pharmaceutical industries. Plants provide a vast array of secondary metabolites against environmental stress or other factors like nest attacks, wounds, blood boosting and injuries. The phytochemical properties produced by plants are said to have various therapeutic uses from time immemorial. Plants serve as an essential aspect of human diet supplying the body with minerals, vitamins and salts, certain hormone precursors and of course energy and protein (Onyenuga and Fefuga, 2018). Plant leaves have nutrient and medicinal benefits which make them highly needed in diets (Odoemelam, 2015). Among these are the leaves of *W.lateritia*, popularly known as blood plant.

Morphologically, *W.lateritia* is a flowering plant belonging to the family of *Acanthaceae* that grows wild in the evergreen plantation of East Africa (Bumbari*et al.,* 2009). The leaves have high economic and medicinal use. (Okafor and Okigbo, 2012). The leaves are used in folkloric medicine after boiling and other processes for boosting of blood, treatment of anaemia, liver damage and inflammation (Aja *et al.,* 2016). *W.lateritia* is predominantly found in Sierra Leone but recently has been observed in several parts of the world like Nigeria. Here in Ebonyi state it can easily be obtained in a large number in places like Ivo, Ikwo, Unwana and Izzi Local Government Areas. *W.lateritia* is called "Ogwuobara" in Igbo, "Ogu n' eje" in Yoruba, and "Magnijini" in Hausa language. The rise in the treatment of diseases with herbal medicine is almost universal among developed and underdeveloped countries and often more affordable than purchasing synthetic drugs. Despite the rise of *W. lateritia* leaves for the treatment of various diseases, there is paucity of documented data available. It is therefore very important for us to augment the available information on *W. lateritia* leaf research (Aja *et al*., 2016). Many of the modern medicine in the early history contain vitamins that have a lasting importance to health when consumed by humans and is useful in the effective treatment of human diseases (Aja *et al.,* 2016). Medicinal plants are not different in terms of how they work, herbal plants are becoming more of mainstream as improvement in analysis and quality central along with advantages in clinical research have shown the treatment and prevention of diseases. Herbal plants have been studied and used as alternative treatment for diseases caused by microorganisms, but the full potential of plants remain under exploited. The leaves of *W.lateritia* are purportedly used for blood boosting, and also for treating different ailments in folkloric medicine. The medicinal and nutritional potential available in the plant depends on its chemical composition. The general public is becoming very interested in the plant because of its medicinal and nutritional importance; however, there are little or no documented reports to this regard, it is on this basis that this work was designed. Hence it is pertinent to investigate its anti-anaemic potentials and of course the safe dose. Therefore, information on its phytochemicals, proximate, vitamin and mineral compositions might be of benefits to scientists in drug design. Determination of possible phytochemicals (quantitatively) that may be present in the leaf extracts of *W.lateritia*, LD_{50} determination, determination of liver function parameters (AST, ALP and ALT), and determination of total protein, albumin and globulin in anaemic albino rats administered with aqueous, chloroform and methanol leaf extracts of *W. lateritia.* Next is the determination of total bilirubin and direct bilirubin of the anaemic albino rats.

MATERIALS AND METHODS

Ethical approval

The National Institute of Health (NIH) approved guideline for the care and use of laboratory animals was adopted for this study.

Plant materials

Fresh leaves of *Whitfieldia lateritia* used for this study were collected from Unwana community, Afikpo-North Local Government of Ebonyi State, Nigeria. The leaf was authenticated by Dr Garuba Omosun of the Plant Science and Biotechnology Department of Michael Okpara University of Agriculture; some of the leaves were deposited in the herbarium with authentication number W0045, for reference purposes.

Figure 1: Picture of *W. lateritia*.

Sample preparation

The leaves were destalked, washed with water and shade dried for two weeks at ambient temperature with constant turning to avert fungal growth. The dried leaves were milled to obtained the vegetable leaf meals (VLMs) using an electric blender and was stored at 4ºC temperature in refrigerator in well labeled air – tight containers for analysis.

Extraction

The 10% methanol, chloroform and aqueous extracts of the leaves were carried out according to the methods describe by Cowan, (1999). Exactly 400g of powdered leaves was macerated in a stoppered container with the solvents and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter dissolved. The mixture was then strained, the marc (the damp solid material) was pressed, and the combined liquids were clarified by filtration or decantation after standing. Therefore aqueous, chloroform and methanol, are the solvents used for extraction and were chosen on the basis that; These solvents will maximize the yield of the active compounds and minimize extraction of unwanted compounds in the crude extract. Secondly the active compounds will be soluble in the solvents. Again the nature of polarity of therapeutic values will maintain its potency in the solvent. Finally, it is relatively safe to use these solvents even when subjected to heat. Exactly 400g of the powder was macerated in 4 litres of aqueous solution for 24hrs. The extract was then sieved with cheese cloth and later filtered using buckner funnel and Whatman No1 filter paper. The filtrate was allowed to evaporate to dryness using rotary evaporator and then stored in an air tight sterile container in the refrigerator at 4℃ until required.

Acute Toxicity and Lethal Dose (LD_{50}) test

The acute toxicity study of all extracts of *W. lateritia* leaves was carried out using the modified method of Lorke, (1983). The test was divided into two phases. In the first phase, total of nine randomly selected adult mice were divided into three groups (n=3) and received 10, 100 and 1000 mg/kg b. w. of the extracts, and there were no signs of toxicity and death recorded after 24 hours of observation. The doses for phase two were determined based on the outcome of the phase one. There was no death recorded, a fresh batch of animals were used following the same procedure with higher doses of 1600, 2900, and

5000 mg/kg body weight of the extract. The animals were observed for 24 hours for signs of toxicity and death. The LD_{50} was calculated as the geometric mean of the high nonlethal dose and lowest lethal dose (Lorke, 1983).

Induction of Haemolytic Anaemia with 2,4 DNPH and collection of blood samples.

A modified method described by Berger (1980), was used in this study. The animals of Groups II to VI received 2,4-dinitrophenylhydrazine (20 mg/kg body weight) once daily for seven days. On the eighth day, their blood samples were collected by tail snip of each rat into heparinized capillary tubes for haematological analysis. The tails were first sterilized by swabbing with 70% ethanol and then the tip of the tails pierced. Bleeding was enhanced by gently milking the tail from the body towards the tip. Blood of approximately 2 ml was drawn into heparinized capillary tubes containing anticoagulant for haematological parameters analysis. Rats with packed cell volume (PCV) less or equal to 30 (≤30) were considered anaemic and selected for the experimental groups. On the twenty-first day the animals were euthanized by use of chloroform and blood was collected through cardiac puncture for further laboratory experiments.

Experimental Design

A total of 36 healthy rats were used for the experimental study, they were randomly allotted into six (6) groups (I to VI) with 6 animals per group (n=6). Appropriate solvent (10% tween 80) vehicle was used to dissolve the extracts. All test substances were administered once daily for 21 consecutive days by oral-feeding cannula. All tested substances were prepared fresh before administration through oral gavage according to design below:

Group I: Normal control: non-anaemic rats administered 7 ml/kg b. w. of 10% tween 80.
Group II: Negative control: 2,4-dinitrophenylhydrazine-induced anemic rats administered 7 ml/kg b. w of 10% tween 80.

Group III: Positive control: 2,4-dinitrophenylhydrazine-induced anemic rats administered 0.6 ml/kg b. w of multivitamin Astifer.

Group IV: Test Group I: 2,4-dinitrophenylhydrazine-induced anemic rats administered 400 mg/kg b. w. of aqueous leaf extract of *W.lateritia*.

Group V: Test Group II: 2,4-dinitrophenylhydrazine-induced anemic rats administered 400 mg/kg b. w. of chloroform leaf extract of *W.lateritia.*

Group VI: Test Group III: 2,4-dinitrophenylhydrazine-induced anemic rats administered 400 mg/kg b. w. of methanol leaf extract of *W.lateritia.*

Phytochemical analysis

Selected phytochemical determination was carried out on extracted leaf samples using standard methods of AOAC (2010) except for flavonoid and alkaloid which were determined as described by Harborne (1993).

Biochemical Analysis

The following parameters were determined in blood samples collected by ocular puncture:

Serum globulin using the method described by Ochie and Kolhatkar (2000), serum Albumin using the method described by Trease and Evans (1989) and total protein using the method described by Ochie and Kolhatkar (2000).

Assay of serum alanine aminotransferase (ALT) activity

The ALT substrate and phosphate buffer, 0.5ml each were pipetted into two sets of test tubes labelled B (Sample blank) and T (sample test) respectively. The serum (0.1ml) sample was added to the sample test (T) only and mixed properly: then incubated for exactly 30 min in a water bath at 37^0C. A volume, 0.5ml each of 2,4-dinitrophenyl hydrazine was added to both the test tubes labelled T (sample test) and B (Sample blank) immediately after the incubation. Also, 0.1ml of serum sample was added to the (B sample blank) only. The entire medium was mixed thoroughly and allowed to stand for exactly 20min at 25^0C. After that, 5.0 ml each of sodium hydroxide (NaOH) solution was added to both test tubes and also mixed thoroughly. Absorbance of the sample (A_{sample}) against the

sample blank was read at a wavelength of 456nm after 5 min.

Assay of serum aspartate aminotransferase (AST) activity.

The method of Reitman and Frankel (1957) was used. The AST substrate and phosphate buffer, 0.5ml each, were pipette into the sample blank (B) and sample test (T) test tubes respectively. The serum sample, 0.1ml was added to the sample test (T) only and mixed immediately. It was then incubated in a water bath for exactly 30min at 37°C. A volume, 0.5ml of 2,4-dinitrophenyl hydrazine was added to both sample blank (B) and sample test (T) test tubes immediately after incubation. Also, 0.1ml of the sample was added to the sample blank (B) only. The medium was mixed and allowed to stand for exactly 20min at 25^0C. Finally, 5.0ml of sodium hydroxide (NaOH) was added to both the sample blank (B) and sample (T) test tubes and mixed thoroughly. The absorbance of sample (A_{sample}) was read at a wavelength of 546nm against the sample blank after 5mins.

Determination of total and direct bilirubin of the colourimetric method

To determine total or unconjugated bilirubin (Tb)**,** two separate cuvettes labelled SB (sample blank) and S (sample) were set up. A volume of 0.2ml of reagent 1 was pipette into the sample blank and the test sample cuvettes respectively. A drop of reagent 2 was added into the test sample (S) cuvette. Also, 1ml of reagent 3 was added to the two cuvettes labelled SB and S. the sample (0.20ml) was added to the two cuvettes which were mixed thoroughly and allowed to stand for 10 minutes at 25^0C then the absorbance of the sample against the sample blank was read at a wavelength of 560nm.

Procedure for direct or conjugated bilirubin (Db)

Two separate cuvettes labelled SB (sample blank) and S (sample) were set up. A volume of 0.20ml reagent 1 was pipette into the sample blank and the test sample cuvettes respectively. A drop of reagent 2 was added into the test sample(S) cuvette. Also, 2ml, of sodium chloride (9g/L) each was added to the two cuvettes labelled SB and S. the sample 0.20ml each was added to the two cuvettes which were mixed thoroughly and allowed to stand for 10 minutes at 25^0C then the absorbance of the sample against the sample blank (A_{SB}) was read at a wavelength of 650nm.

Calculation:

Total or unconjugated bilirubin (μmol/L) = 185 x A_{TB} (578nm)

Total or unconjugated bilirubin (mg/l) = 10.8 x A_{TB} (578nm).

Direct or unconjugated bilirubin (μmol/L) = 246 x A_{DB} (546nm).

Determination of serum alkaline phosphatase (ALP)

The procedure of Plummer (1971) was used. The serum sample (0.5ml) was pipetted into the sample test tubes (T) and 0.5ml of the standard was pipette into the sample blank (B) respectively. One ML (1ml) of distilled water was added to the sample test tubes (T) and sample blank (B).

A drop of phenolphthalein monophosphatase was also added to both sample blank (B) and sample test (T) and mixed immediately; then incubated into a water bath for exactly 20 minutes at 25^0C. A volume of 0.5ml of 2-amino-2 methyl-1propanol was added to both sample blank (B) and sample test tubes (T) immediately after incubation. Then the absorbance of the (A_{sample}) was read at a wavelength of 550nm against the sample blank after 5min.

Statistical analysis

The data obtained from the laboratory test were subjected to one way analysis of variance (ANOVA). Differences between means at $P < 0.05$ were accepted as significant. The results were expressed as mean standard deviation (SEM). This analysis was estimated using Statistical Package for Social Science (SPSS), version 17 and represented with appropriate charts.

RESULTS

Quantitative phytochemical tests of W. lateritia leaves showed that it contained some phytochemicals namely phenol, tanninins,

flavonoids, terpenoids, steroids, glycosides, reducing sugars and alkaloids. Sponini was not detected (Table 3).

Result of acute toxicity

Acute toxicity tests on *W. lateritia* in albino rats established a high LD_{50}, which suggests that the aqueous, chloroform and methanol extracts of the leaf *W. lateritia* may be generally regarded as safe with a remote risk of acute intoxication and sedation at high dose above 5000mg/kg b.w. The result is as shown in Table 4.

Table 3: Quantitative phytochemical tests of *W. lateritia* leaves

Phytochemicals	Composition (mg/g) plus/minus SEM
Phenol	2596.533 ± 11.856
Tannins	18.687 ± 0.782
Flavonoids	72.750 ± 14.356
Terpenoids	5.197 ± 1.260
Steroids	0.577 ± 0.158
Glycosides	4.413 ± 0.041
Reducing Sugar	542.710 ± 14.911
Saponins	ND
Alkaloids	1426.120 ± 35.523

Key: ND = Not Detected

Table 4: Acute toxicity of aqueous, chloroform and methanol extracts of *W. lateritia* extract

	Dosage (mg/kg b.w)	Mortality		
Phase 1		Aqueous extract	Chloroform extract	Methanol extract
Group 1	10	0/3	0/3	0/3
Group II	100	0/3	0/3	0/3
Group III	1000	0/3	0/3	0/3
Phase II				
Group 1	1600	0/3	0/3	0/3
Group II	2900	0/3	0/3	0/3
Group III	5000	0/3	0/3	0/3

$LD_{50} = \sqrt{D_0} \times D_{100}$, D_0 = Highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality. Thus, in each extract, D_0 = 5000 and D_{100} = Nil

Mean protein, albumin and globulin concentrations of 2, 4-dinitrophenyl-hydrazine induced anaemic rats treated with *W. lateritia* leaf extracts

Figure 2 revealed that the total protein, albumin, and globulin concentration of 2, 4-dinitrophenylhydrazin induced anaemic rats administered 10% tween 80 were significantly ($P < 0.05$) lower compared with the normal control rats. The anaemic rats treated with 0.6 ml/kg b. w. of astifer showed significant ($P < 0.05$) increase in total protein, albumin and globulin concentration compared with the anaemic rats administered 10% tween 80. Anaemia induced rats treated with 400 mg/kg b. w. of aqueous and methanol extracts of *W. lateritia* leaf showed significant ($P < 0.05$) reduction in total protein and albumin concentration compared with anaemic

rats treated with 0.6 ml/ kg b. w. of astifer, while the chloroform extract treated anaemic rats showed non-significant ($P > 0.05$) reduction compared with astifer treated anaemic rats. Anaemic rats treated with 400 mg/kg b. w. of chloroform and methanol extracts showed significant ($P < 0.05$) increase in total protein, albumin and globulin concentration compared with the anaemic rats treated with 400 mg/kg b. w. of aqueous extract of the leaf. Group I: normal control; Group II: negative control; Group III: administered 0.6 ml/kg body weight (b.w) of Astifer (standard Haematinic); Group IV to VI were administered 400 mg/kg b. w. of the aqueous, chloroform and methanol leaf extracts, respectively.

Mean liver marker enzyme activities of 2, 4-dinitrophenylhydrazine induced anaemic rats treated with *W. lateritia l*eaf extracts

The alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities of anaemic rats administered 10% tween 80 showed significant ($P < 0.05$) reduction compared with normal control rats. The rats treated with 0.6 ml/kg b. w. of astifer after 2, 4-dinitrophenylhydrazine anaemia induction showed significant ($P < 0.05$) increase in ALT, AST and ALP activities compared with anaemic rats administered 10% tween 80. Anaemic rats treated with 400 mg/kg b. w. of aqueous extract of *W. lateritia* leaf showed significant ($P < 0.05$) increase in ALT and AST activity compared with astifer treated anaemic rats. The rats treated with 400 mg/kg b. w. of chloroform and methanol extracts of *W. lateritia* leaf after anaemia induction showed non-significant ($P > 0.05$) reduction in AST, ALT and ALP activities compared with anaemic rats treated with 400 mg/kg b. w. of aqueous extract of the leaf as shown in Figure 3.

Mean Bilirubin Concentration of 2, 4-dinitrophenylhydrazine induced anaemic rats treated with *W. Lateritia* leaf Extracts

The total bilirubin concentration of 2, 4-dinitrophenylhydrazine induced rats administered 10% tween 80 was non-significantly ($P > 0.05$) higher compared with the normal control rats. Astifer treated anaemic rats at 0.6 ml/kg b. w showed non-significant ($P > 0.05$) reduction in total bilirubin concentration compared with anaemic rats administered 10% tween 80. Anaemia induced rats treated with 400 mg/kg b.w. of aqueous and methanol extracts of *W. lateritia* leaf showed non-significant ($P > 0.05$) reduction in total bilirubin concentration compared with anaemic rats treated with chloroform extract of the leaf. The direct bilirubin concentration of anaemia induced rats administered 10% tween 80 showed significant ($P < 0.05$) reduction compared with normal control rats. Anaemic rats treated with 0.6 ml/kg b. w of astifer and 400 mg/kg b. w. of extracts of *W. lateritia* leaf showed non-significant ($P > 0.05$) increase in direct bilirubin concentration compared with anaemic rats administered 10% tween 80. The anaemic rats treated with aqueous and methanol extracts of *W. lateritia*leaf showed non-significant ($P > 0.05$) increase in direct bilirubin concentration compared with astifer treated anaemic rats as shown in Figure 4.

DISCUSSION

Anaemia is a well-known life-threatening condition. It may be caused by excessive blood loss, haemolysis, and deficiency associated with RBC synthesis (due to iron deficiency). The synthetic drugs available for its management/cure are not without their side effects, they may also be inaccessible and costly to some rural sufferers. All these limitations of the synthetic drugs have necessitated researches, focusing on less toxic herbal therapies known for their anti-anaemic efficacies as claimed by ethno medicinal practitioners. Phytochemicals are plant

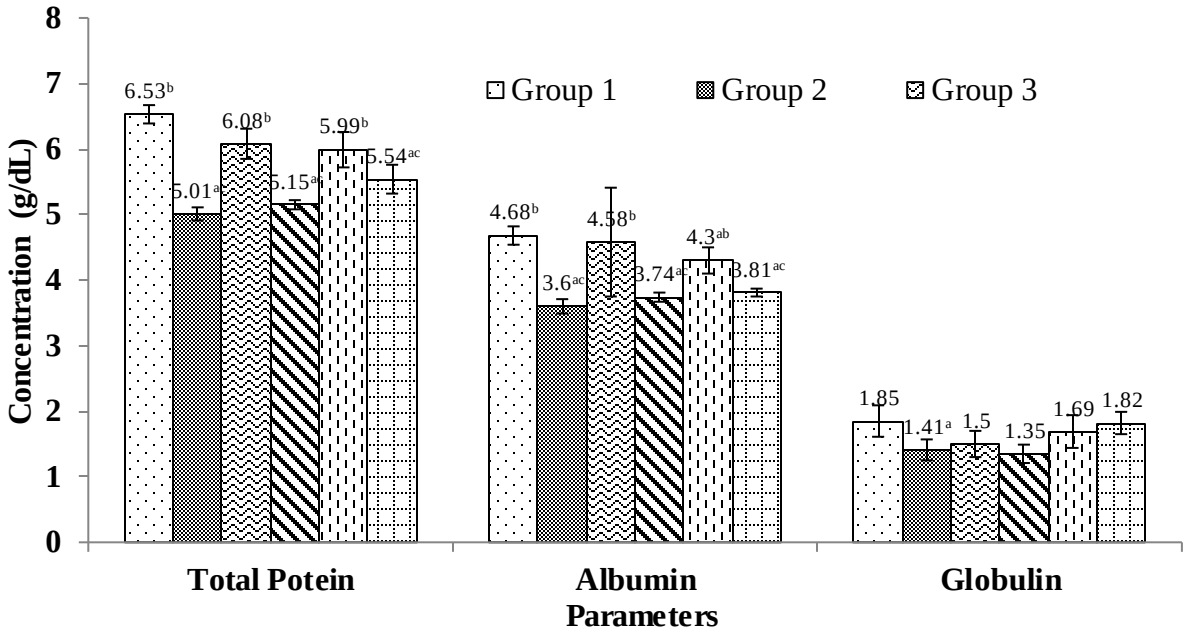

Fig. 2: Effects of *W. lateritia* leaf extracts on serum protein, albumin and globulin concentrations

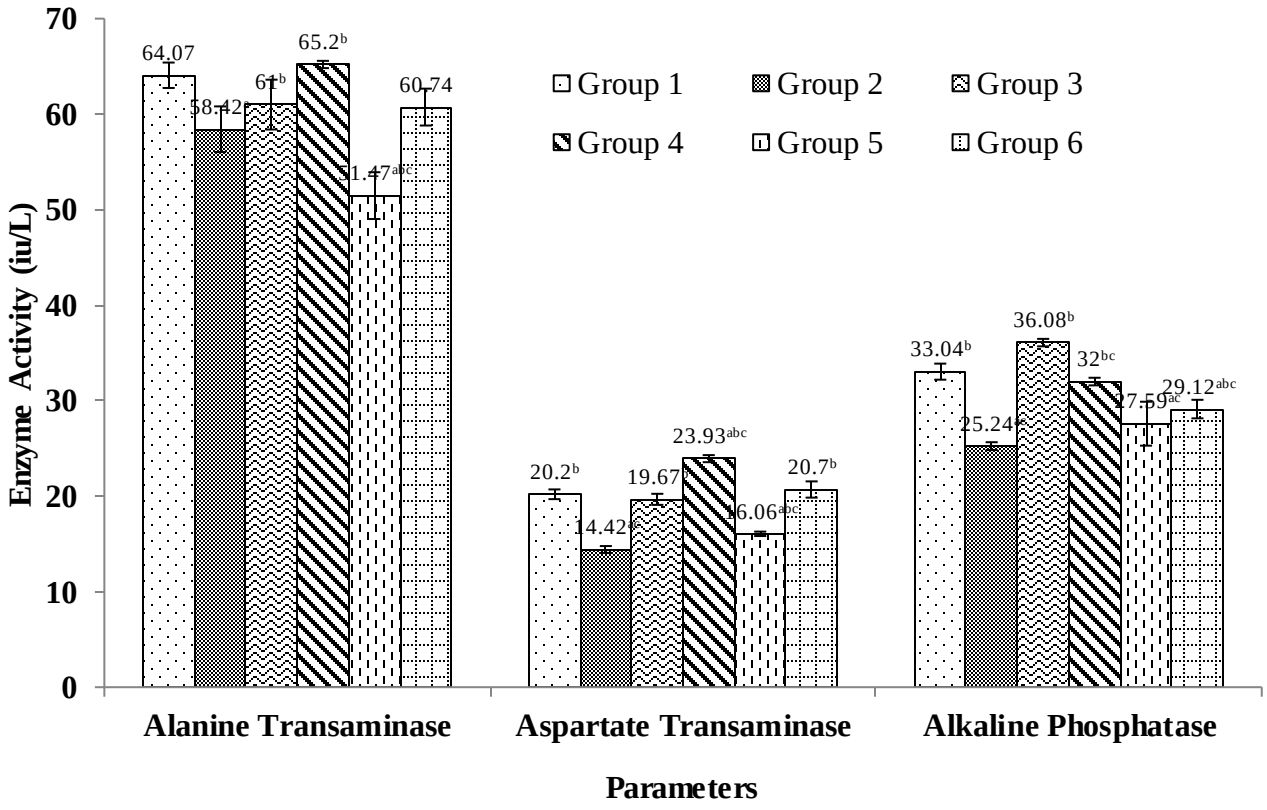

Fig. 3: Effect of *W. lateritia* leaf extracts on selected liver enzyme activities

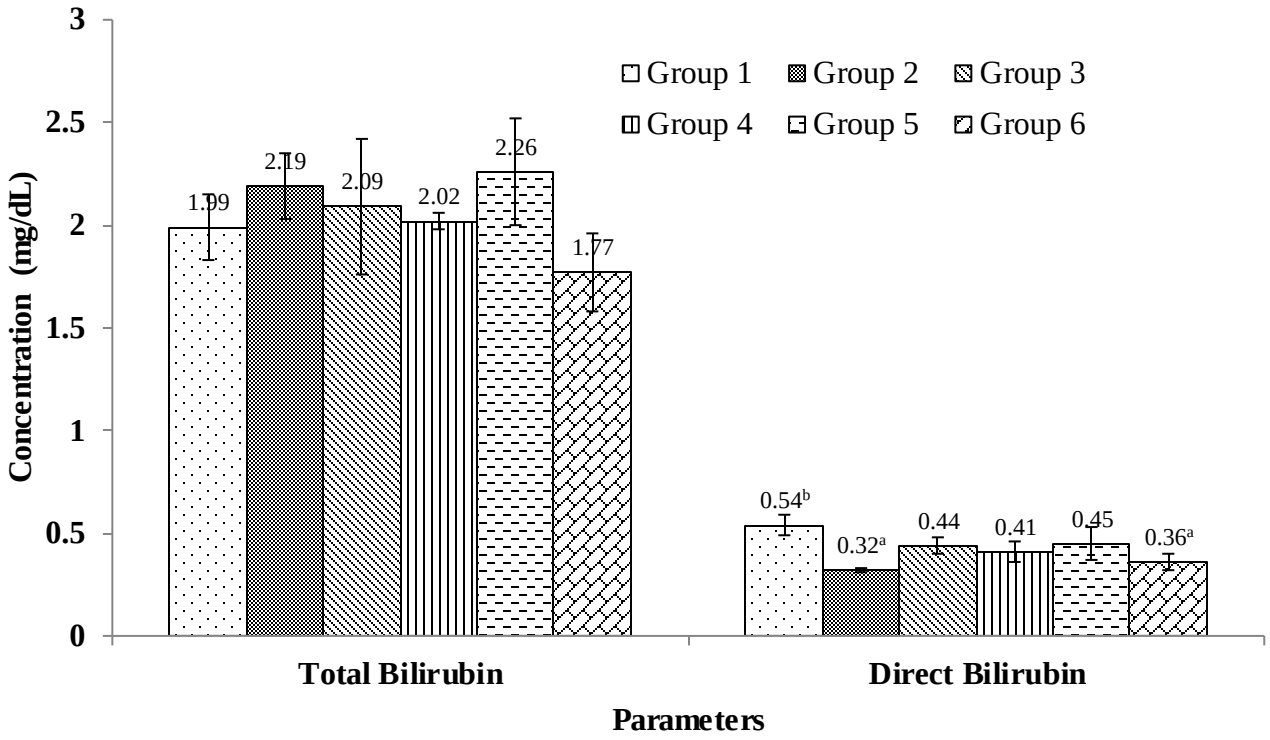

Fig. 4: Effects of *W. lateritia* leaf extracts on bilirubin concentration

derived chemical compounds that can be used as therapeutic agents. They are responsible for different colours, flavours, smell and other organoleptic properties (Eleje and Akujobi, 2011). The results of the quantitative phytochemical analyses of *W. lateritia* are shown in Table 3. It is obvious from the result that the leaves are rich in phenols, tannins, flavonoids, reducing sugars, and alkaloids. The presence of these biologically active compounds suggest that plants could serve as potential sources of drugs and their secondary metabolites could exert some biological activities when taken by animals (Edeoga *et al*., 2005). Epidemiological studies have shown that high consumption of vegetables is associated with a lowered incidence of anaemia, liver disease, heart disease, inflammation, arthritis, neurodegenerative diseases and other immune related diseases (Krishna *et al.,* 2014). Flavonoids have been shown to augment humoral response by stimulating the macrophage and B-lymphocytes involved in antibody synthesis. Reports indicate that several types of flavonols stimulate human peripheral blood leucocyte proliferation (Sharifi*far et al.,* 2009) and possess antibacterial, anti-inflammatory, anti-allergic, anti-viral, hepato-protective and antineoplastic activities. Flavonoids also function as reducing agents, free radical scavengers and quenchers of singlet oxygen formation (Kayode and Kayode, 2011), thus very useful in counteracting the free radicals generated by immune depressants. The alkaloid content or composition was appreciably high. Alkaloids being a class of secondary metabolites and with their wide range of pharmacological activities including antimalaria, anticancer, antibacterial and antihyperglyceric activities have been of essence in drug design (Edeoga *et al.,* 2005). Tannins have been shown to play major roles as anti-diarrheal and anti-hemorrhagic agent (Edeoga *et al.,* 2005). The terpenoids have also been shown to decrease blood sugar levels in animal studies. Glycosides may be crucial in the transduction of intracellular signals mediated by neurotransmitters, hormones and neuromodulators receptors. When activated, these molecules can act on several intracellular targets (De-Weerd*et al.,* 2007). The immune response of both human and animals may be influenced by several essential nutrients which modify the immune system function. It is generally assumed that many important infections of human and animals have been associated with a nutritional deficiency which generates a suppression of immune response

(Chandra, 1990). Tannin, a class of astringent, is a phenolic biomolecule that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids (Edeoga *et al.,* 2005). Hence the leaf extracts of *W. lateritia* could be of great importance to human health. Acute toxicity tests on *W. lateritia* in albino rats using the method of Lorke (1983) established a high LD_{50}, which suggests that the aqueous, chloroform and methanol extracts of the leaf *W. lateritia* may be generally regarded as safe with a remote risk of acute intoxication and sedation at high dose above 5000mg/kgb. w. Signs of acute toxicity include decreased locomotor activity, decreased feed intake, tremor, change of hair colour, prostration and death (Barbosa-Ferreira *et al.,* 2005). None of these signs was noticed in the experimental mice given the extracts. The degree of safety is also consistent with its popular use locally. Thus, since *W. lateritia* is believed to have hepato-protective potentials by many traditional healers, the experimental determination of this good safety margin would justify the plant as relatively safe at the dose level (400mg/kg b. w) used in this study.

The result for the serum total protein in Figure 2 shows that serum protein was elevated in the control group 1 when compared to the group induced with anaemia and treated with the vehicle tween 80 and leaf extracts. This suggests that induction of anaemia also led to decreased serum total protein as seen in group 2. The treatment however boosted the serum total protein significantly ($P<0.05$) in the group treated with the standard astifer multivitamin, aqueous, chloroform and methanol leaf extracts. This suggests that the chloroform extract had comparative effect with that of the standard drug (astifer). The decrease in the total serum protein in the group induced with anaemia could be as a result of the toxicity induced by 2, 4 DNPH and diminished synthesis of protein by liver (Naom*et al.,* 2008), while the increase in the groups treated with astifer multivitamin, aqueous, chloroform and methanol extract is due to the presence of phytochemical in the plant extract which are well known to have direct influence on the liver and boost protein synthesis as confirmed by Aja *et al*., (2016). This is in line with the study of (Sarkiyayi and Alduirasheed, 2013), who's preliminary investigation on anti-anaemia in *W. lateritia* leaf extracts buttress the assertion. Protein is important for structure formation and repairs of tissue building (Thompson *et al.,* 2013).

Liver function tests (LFTs or Lfs) are groups of blood tests that give information about the state of the liver. The liver is a vital organ that functions in detoxification, storage, and other biochemical metabolisms necessary for the body (Mbuh *et al.,* 2003). This study equally examined the changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), total bilirubin and direct bilirubin activities in induced anaemic albino rats. The change in the mean serum of ALT, AST and ALP are presented in Figure 3 in the result, it shows that ALT was not significant ($P>0.05$) in group treated with 400mg/kg b.w of chloroform when compared with the control group. The result also shows that AST concentration in aqueous treated was significant ($P<0.05$) when compared with the control. The result shows that ALP was significantly ($P> 0.05$) decreased in all the groups when compared to the control except in the astifer treated group. This agrees with a similar study by (Kayode and Kayode, 2011) on fluted pumpkin (another popular blood boosting plant). ALT is purely cytoplasmic catalyzing the transamination reaction (Mauro *et al.,* 2006). The decrease in ALT concentration may be as a result of the administration of the astifer and extracts of *W. lateritia*. Any type of liver cell injury can reasonably increase ALT levels. The changes in the mean serum total bilirubin and direct bilirubin concentration are presented in Figure 4. The result shows that there was a significant increase ($P<0.05$) in mean serum total bilirubin concentration in groups treated with 20mg/kg b. w of tween 80 (vehicle), 0.6 ml/kg b.w of Astifer and 400mg/kgb. w of chloroform extract of *W. lateritia* when compared to the control group, while the result for the mean serum direct bilirubin concentration shows that there was a significant decrease ($P>0.05$) in mean serum direct bilirubin concentration when compared with control group. Unconjugated bilirubin is a breakdown product of heme (a part of haemoglobin red blood cells). This agrees with the findings of Shivaraji *et al.,* (2016), whose work showed that when total bilirubin level exceeds 17µmol/L, it indicates liver disease, when total bilirubin level exceeds 40µmol/ L, bilirubin deposition at the sclera, skin and mucous colour occur (Shivaraji *et al.,* 2016).

CONCLUSION

All the data obtained from this study showed strong preliminary evidence that *W. lateritia* leaf extracts have hepato-protective potentials as proven by several biochemical, and liver marker

enzyme activities.Accordingly, the extract can be used as an effective herbal product for the prevention of hepatic diseases and liver related issues. It is believed to be due to its phytochemicals like glycosides, flavonoids etc. that contributed to its efficiency.

Conflict of interest

Authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

ESI designed the study, wrote the protocol, and supervised the work. AOA performed all the laboratory work. MOJ and APO performed the statistical analysis. UEN managed the analyses of the study. AOA and OCE wrote the first draft of the manuscript. AOA, MOJ and APO performed the literature search. All authors read and approved the final draft of the manuscript.

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