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Sub- acute toxicity studies of *Phyllanthus amarus* on haematological parameters and some plasma enzymes activities in mice

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Abstract: Haematological parameters and plasma enzymesactivities were investigated in mice treated with sub-lethal concentration of ethanol extractof *Phyllanthusamarus*. Twenty healthy male mice weighing between 28- 31g were randomly divided into four groups (A- D) of five rats each.Group A were orally dosedwith 1ml of distilled water, while the other groups (B - D) were administered with 1000, 1500, 2000mg/kg body weight of the extract, after seven days treatment period, the mice were anaesthetised and blood samples collected via cardiac puncture for analysis. All doses of theextract under investigation did not significantly ($P > 0.05$) alter the level of Hb, PCV, RBC, MCH, MCHC, MCV and platelet count, but however induced a significant decrease ($P< 0.05$) in the level of white blood cell,a significance increase ($P< 0.05$) was also observed at the dose of 1500 and 2000mg/kg body weight in AST, ALT, ALP and LDH levels, an indication that sub-acute oral doses of *P. amarus*may compromise immune response and induce organ damage.

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Introduction

Plants have been and are still being processed and utilized as raw materials for pharmaceutical applications. Many of our modern drugs and processed scientific medicines are of plant origin (Treas*et al.,* 2001). Man has been using herbs for the treatment of many diseases and the plant *Phyllanthusamarus* is one of such plant commonly used in Nigeria.*P.amarus*is a plant that belongs to the family Euphorbiaceae and of the genus *Phyllanthus* and the species is *amarus* and has about approximately 800 species which are found in tropical and subtropical countries of the world (Mazumder*et al.,* 2006; Tahseen*et al.,* 2013).*P. amarus*is a plant with many reported medicinal properties and broad spectrum of pharmacological activities including antiviral, antimicrobial, anti-plasmodial, anti-inflammatory, anticancer, anti-diabetics, antioxidant and diuretics properties among others (Nyveldt*et al.,* 2001). There is a belief that because herbal remedies are derived from nature they are therefore devoid of adverse or toxic side effects. Hence, the toxicity of herbal medicine is often not assessed as such, the users often look at the medicinal benefit of the plant and neglecting their toxic effects to organs and tissues. Toxicity is the degree to which a substance can damage an organism such as animal, plant, bacterium as well as substructure of the organism such as a cell (cytotoxicity) or organ such as liver (hepatotoxicity). A central concept of toxicity is that the effects are dose–dependent. The extent to which an organ is susceptible to toxicity varies from organ to organ. For example the kidneys and liver are more highly vascularised making them more susceptible to toxicity than the bone tissues. Blood which forms the main medium of transport in the body is a very important tissue. It serves to transport many drugs and xenobiotic. Since all foreign compounds are distributed via the bloodstream, the various components, cellular and non-cellular, are initially exposed to significant concentrations of toxic compounds (Timbrel, 2009). Some plant materials when ingested either in the raw state or their extract have been reported to cause anaemia which may result from sequestration of red blood cell in the spleen, impaired red cell production or primary bone

marrow dysfunction (Bin-Jaliah *et al*., 2014). Damage to and destruction of the blood cells results in a variety of consequences such as a reduction in the oxygen-carrying capacity of the blood if the cells affected are the red blood cells. The assessment of blood is relevant to the evaluation of risks since the haematological system carries a higher predictive value for toxicity in humans when the data are extrapolated from animal studies (Majeed*et al.,* 2015).

Due to the widespread usage of *P. amarus*in traditional medicine; this study seek to assess the sub-acute toxicity of ethanol extract of *P. amarus*for safety or possible toxic effects using changes in haematological parameters and plasma enzymesactivities as indices of toxicity in mice and it's expected that the findings from this work may add to the overall value of the medicinal potential of the plant.

Materials and Methods

The plant sample were harvested behind the Faculty Building (FB1) Laboratory of the Federal University Otuoke, Ogbia Local Government Area of Bayelsa State, Nigeria and was identified and confirmed by the Plant Science section of Biology department, Federal University Otuoke, Bayelsa State.

Preparation of plant extract.

The whole plant material (*P. amarus*) was thoroughly washed with distilled water to remove debris and contaminants, it was then air dried for 14days so as to give a constant weight, and then pulverized using an electric blender (Blender 462 Nakai Japan). 100 g of the powdered *P.amarus*was extracted in 300ml of absolute ethanol for 24 hours at room temperature with constant shaking using a flask shaker (Denly A-500). The extract was filtered with WhatmanNo.1 filter paper and the resulting filtrate evaporated to dryness using a rotary evaporator at 40℃ to give 3.15 g of crude extract.

Experimental Animals and Design

The twenty (20) adult male albino mice weighing between 28-31g were randomly divided into four groups [A- D], with five mice in each group.Group A: (Control): Received 1.0ml of distilled water,group B: received 1000mg/kg body weight of extract,group C: received 1500mg/kg body weight of extract,group D: received 2000mg/kg body weight of extract.Administration of ethanol extract of *P. amarus* was performed orally once daily 8:30am ± 30mins using an oral cannula attached to a 2ml syringe. The mice were fed *ad libitum* with grower's mash and tap water.The study was carried out for 7 days and on the 8th day the animals were sacrificed. The mice were however allowed to acclimatize for a period of 7 days before extract administration was commenced, and all protocols were performed in accordance with Institutional Animal Ethical Committee (IAEC) as per the directions of the Committee for the Purpose Of control and Supervision of Experimental Animal (CPCSEA).

Method of collection of blood and serum sample.

Blood samples was collected via cardiac puncture using a 2ml syringe from each mouse and placed into separate EDTA container for analysis of haematological parameters and into plain containers for clinical biochemistry assay. For biochemical analysis, the blood samples in the plain test tubes were allowed to stand for 3 hours for complete clotting and then centrifuged at 5000 rpm for 15 minutes using a centrifuge. The serum was withdrawn using an automated pipette and transferred into other clean vial.

Haematological analysis and liver enzymes assay

Determination of haemoglobin concentration, packed cell volume, white blood cell, mean cell haemoglobin concentration, mean cell volume, mean cell hemoglobin, platelets, red blood cell count were determined using methods described by Cheesbrough(2006).The following liver function test were conducted to investigate derangement in the liver of the animals used for the study, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were determined by colorimetric method as described by Ochei and Kolhartkar, (2008), using commercial assay kit from Randox Laboratories Ltd, Co. Antrim, United Kingdom.

Data analysis:

The statistical package for social sciences (SPSS) computer software version 16 was used for data analysis. The results were expressed as mean of replicate determinations ± standard deviation (S.D), results were analysed by using one way analysis of variance (ANOVA). $P \leq 0.05$ was regarded as significant.

Results: Table 1: shows the effect of *P.amarus* on some haematological parameters in the experimental animals. Theresult indicated a non-significant increase ($p > 0.05$) in the PCV, Hb, RBC, Platelet, MCH, MCHC, MCV, and MCHC in all treatment groups when compared to control. There was however a significant depletion ($p<0.05$) in the level of total WBC in the treated group when compared to control group.

Table 1: Effects of *P. amarus*treatment on haematological parameters of mice

Haematological Parameters	Extract Dose mg/kg			
	Control	1000	1500	2000
PCV (%)	41.00± 2.35[a]	40.00 ± 3.21[a]	40.00 ± 2.74 [a]	41.00 ± 3.08 [a]
Hb (g/l)	141.60 ± 6.39 [a]	139.00 ± 7.31 [a]	138.60 ± 8.08 [a]	140.20 ± 9.20 [a]
RBC ($\times 10^{12}$/L)	6.58 ± 0.28 [a]	6.56 ± 0.27 [a]	6.54 ± 0.23 [a]	6.54 ± 0.15 [a]
Platelet ($\times 10^{9}$/ L)	237.40 ± 5.03 [a]	238.20 ± 4.66 [a]	239.80 ± 6.69 [a]	239.00 ± 6.78 [a]
WBC ($\times 10^{9}$/L)	8.86 ± 0.26 [a]	8.30 ± 0.41[b]	8.02 ± 0.33[c]	7.40 ± 0.44[d]
MCH (pg/cell)	25.40 ± 0.96 [a]	25.40 ± 0.96 [a]	25.40 ± 0.96 [a]	25.40 ± 0.96 [a]
MCV (fl)	33.60 ± 2.07 [a]	33.60 ± 2.07 [a]	33.60 ± 2.07 [a]	33.60 ± 2.07 [a]
MCHC (%)	77.60 ± 2.07 [a]	77.60 ± 2.07 [a]	77.60 ± 2.07 [a]	77.60 ± 2.07 [a]

Values are mean of five replicates ± standard deviations, values having different superscript in the same columnvaries significantly at P<0.05.

Table 2: Indicates the plasma enzymes activities of mice when administered with ethanolic extract of *P.amarus*. The values obtained showed that the AST activities was not significant altered (*p> 0.05)*by the 1000mg/kgdose of the extract but was increased significantly (*p < 0.05*) by the oral administration of 1500 and 2000mg/kgdose of the extract. The level of ALT did not show a significant increase(*p> 0.05)* in mice administered with 1000 and 1500mg/kg body weight of the extract,conversely the 2000 mg/kg dose of the extract significantly (p<0.05) increased the activities of ALT. ALP and LDH activities increased significantly in mice administered with 1500 and 2000mg/kg when compared to control,however there was no significant increase when 1000mg/kg dose was administered to the mice.

Table 2: Effects of *P. amarus*treatment on plasma enzyme activity of mice

Plasma enzyme activity	Extract Dose mg/kg			
	Control	1000	1500	2000
AST (U/L)	56.80 ± 2.39 [a]	58.00 ± 2.74 [a]	60.40 ± 3.05 [b]	69.60 ± 2.30[c]
ALT (U/L)	39.60 ± 2.70 [a]	38.00 ± 3.08 [a]	42 60 ± 2.30 [a]	44.80 ± 3.70 [b]
ALP(U/L)	61.00 ± 2.92 [a]	60.00 ± 2.55 [a]	66.20 ± 3.03 [b]	69.20 ± 3.56 [c]
LDH (IU/L)	184.60 ± 3.65 [a]	186.20 ± 4.66 [a]	196.80 ± 1.33 [b]	202.00 ± 4.53 [c]

Values are mean of five replicates ± standard deviations, values having different superscript in the same column varies significantly at P<005.

Discussion

Blood is known to be the most important body fluid that regulates various vital functions of the body including transport of metabolic substances and defense against foreign substance. Blood act as a pathological reflector of the status of exposed animals to toxicants and other conditions and/or agents (Olafadehan, 2011). Assessment of haematological parameters can be used to explain haematological functions of a chemical compound or

plant extracts in an organism and further provides information regarding the status of bone marrows activity and haemolysis. It can also be used to determine the extent of deleterious effect of foreign compounds including plant extract on blood constituents of an animal (Iniaghe*et al*., 2013). In this study, the ethanol extract of *P.amarus* did not demonstrate varying degrees of changes in the red blood cells and their indices (MCV, MCH, MCHC, PCV Hb) of normal mice at the dose levels of 1000, 1500 and 2000 mg/kg body weight. MCH, MCV, MCHC relates to individual RBC while Hb, RBC, PCV are associated with the total population of red blood cells. The non-significant change in PCV indicates that the amount of red blood cells present in the mice remained fairly constant. Similarly, the extract did not cause a drop in the haemoglobin concentration thus the oxygen carrying capacity of the blood of animals was not detrimentally affected by the extract administration at the various dose levels. Thus there was no destruction of matured RBCs, bleeding, anaemia and inhibition in blood cells synthesis or bone marrow suppression by any of the active constituents in the extracts This result is in agreement with Bakare*et al.* (2015) who reported that this plant extract does not have the potential to stimulate erythropoietin release in the kidney which is the humoral regulator of RBC production. AlsoUjah*et al*. (2015) also reported a significant increase in PCV, Hb, WBC, MCHC, MCV and PLT when wistar rats induced with arsenate hepatic cell damage were administered 500mg/kg ethyl acetate root extract of *P. amarus* showing that *P. amarus* root has hematopoietic property.

Oyewo*et al*. (2012) reported that the administration of leaf extract of *P.amarus* had no significant effect (*p> 0.05)*on packed cell volume but reported an increase in haemoglobin count significantly ($p< 0.05$) at 500 and 1000mg/kg, he also observed a significant reduction in MCH, at 1000mg/kg body weight only and a reduction in mean cell haemoglobin concentration (MCHC) at 500 and 1000mg/kg. The white blood cell which is known to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response showed a significant reduction ($P < 0.05$) in a dose dependent manner hence *P.amarus* has the potential to cause leucopenia. This shows that sub-acute administration of this extracts may lead to a decrease in the principal function of white blood cells, which is to defend against invading organisms,this report contradict the study of Taiwo*et al.* (2009) who reported an increase in WBC at both the lower (100mg/kg) and the higher (400mg/kg) doses. The increase in WBC indicates that *P.amarus* has phytochemicals with the ability to boost the immune system through increasing the population of defensive white blood cells (Bakare*et al*., 2015). The decrease in white blood cell may have consequential effects on the immune system and phagocytic activity of the blood cells of animals. The platelet counts were also not adversely affected indicating that the plant extract also did not affect the production of platelets nor induced thrombocytopenia this result is in line with Bakare*et al.*(2015).

The liver plays a vital role in bio-transformation and sometimes clearing (detoxification) of chemicals that are susceptible to the toxicity. Certain medicinal agents in overdoses and sometimes even at therapeutic ranges may injure the liver (Singh *et al*., 2012).Similarly, kidneys are routinely exposed to high concentrations of medications or their metabolites because their intrinsic functions are to metabolize, concentrate, and excrete compounds.

Many dietary supplements have been associated with nephrotoxicity, either as a direct toxic effect, or secondary to liver dysfunction (Thomson *et al.*, 2002). Damage to these organs often results in elevation in clinical biochemistry parameters such as plasma enzymes; Aspartate aminotransaminase, Alanine aminotransaminase, Alkaline phosphatase and Lactate dehydrogenase. ALT and AST are present in liver and other tissues like muscle and heart and it is particularly useful in measuring hepatic necrosis. They are normally present at low levels in the blood so damage to these tissues would result in the leakage of these enzymes into the blood and cause increase in their levels. LDH is present in various tissues like the liver, heart, kidney and muscles. Increase in this enzyme is seen in damage to these tissues. At the doses of 1500 and 2000mg/kg showed a significant increase in LDH activity which is an indicator of liver damage since there is an increase in the level of ALT, AST and ALP due to the administration of *P. amarus.* This report is in contrast with Sirajudeen*et al.* (2006) who reported a non-significant change in the level of ALP, ALT, AST and LDH at 400 and 800 mg/Kg body weight for 6 weeks.

Conclusion

This study has revealed that sub-acute administration of the ethanolic extract of *P.amarus* at both 1500 and 2000mg/kg can induce a significant increase($p< 0.05$) in the activity of plasma enzyme suggesting that this plant extract induces organ toxicity at a dose dependent level and the significant reduction seen in the total white blood cell count at all dosesindicates that *P.amarus* has the potential to compromise immune function. As good as the medicinal benefits of this plant, low or moderate doses such as ≤1000mg/kg body weight can be employed so as to maximize its potentials, thereby lowering the toxic effect to the barest minimum.

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