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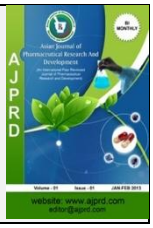

Open Access Research Article

Effect of Ethyl Acetate Fraction of *P. amarus* Leaf on Hematological and Biochemical Parameters in Albino Rat with Arsenic Induced Toxicity

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A B S T R A C T

Therapeutic effect of ethyl acetate fraction of *P. amarus* leaf was probed on hematological and biochemical indices in albino rats induced with arsenic poison. Acute toxicity of 100% ethyl acetate fraction was evaluated at dose range of 100 to 5000mg/kgbw for 24hrs and no apparent signs of toxicity were observed. However, exposure for 10days, death was observed for dose of 1000mg/kg and above. Thus LD_{50} of sub-acute toxicity is 707.11mg/kg. Significant decreased in hematological indices observed, indicate alterations that affect the physiology and hematopoiesis of blood by arsenate administered and with normal levels of MCV and MCH, this suggest normocytic anemia. Furthermore, decrease level of HGB could implies that arsenic might have affected the incorporation of hemoglobin in the red blood cells or the morphology and osmotic fragility in red blood cells and this can affect oxygen carrying capacity of blood, although with MCV and RDW both normal, the RBCs are likely the same normal size. Falls in Platelets count with low MPV and irregular morphology as indicated by PDW infer that the bone marrow isn't producing enough new platelets which may result in low clotting capacity of blood. This is not caused by infection from bacteria as procalcitonin (PCT) in all groups showed no significant difference but the effect was that of arsenic poison. WBCs drop significantly, suggesting susceptibility to opportunistic infections. Result also showed increased bilirubin, an indication of cholestasis. However, these effects were significantly ($P<0.05$) ameliorated in the treatment groups, as levels of these parameters were reversed. Conclusively, this might be as a result of phenolics in *P. amarus*. Phenolics excite their mechanism of therapeutics either by ways of antioxidant activities as free radical scavengers or chelators of metal ions.

Keywords: Hematology, *P. amarus* leaf, Therapeutic, Toxicity, Ameliorate

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INTRODUCTION

To elucidate the therapeutic, nutritive and or toxic effect of medicinal plant during preclinical trials, it is critical to assess the hematological indices in animals. This is because, according to Olson et al. [1] and Ajayi et al. [2] hematological responses to botanicals have higher predictive values for humans when the data are translated from animal studies. And since blood is a route of xenobiotic transport, a rise or fall in hematological variable could suggest excess or suppressed hemopoiesis. Thus the blood tissue is the first point of call in therapeutics and toxicity. Exposure to toxic chemicals like arsenic whether intended or unintended, has been reported to induce changes in blood parameters that are indicative of hematological disorders such as anemia [3], neutropenia, thrombocytopenia and malignancies such as leukemia, lymphoma and myeloma [4]. According to Shen et al. [5], arsenic(As) is a sulphydryl-reactive chemical which is capable of binding and cross-linking cellular proteins, which in turn alters numerous cellular pathways involving suppression of cell cycle checkpoint proteins, decreasing immune surveillance, promotion of apoptosis, expression of growth factors, inhibition of DNA repair and increasing

oxidative stress. The toxicant (As) is present in different forms in the environment and its toxicity relies upon its chemical forms and oxidation states (arsenite AS^{3+}, AS^{3-} or arsenate$AS^{5+}S^{5-}$)[6]. It can be found either asorganic and inorganic forms, of which the inorganic form (AS^{3+}, AS^{5+}) is highly toxic and mobile in the environment compared to the organic form (AS^{3-}, AS^{5-}). Arsenic is ranked first in a list of 20 hazardous substances by the Agency for Toxic Substances and Diseases Registry as reported by John et al. [7]. This hazardous substance is currently poisoning tens of millions of people worldwide of which greater percentage are in the rural communities in developing countries like Nigeria and this people use plant materials for the management of the toxicity related cases, which is without recourse to the hematological effects.

The plant under investigation has been used in folk remedies as blood purifiers and to improve immune. The boiled leaves are considered to be diuretic and therefore used in treating diabetes, dysentery and menstruation [8]. Ita, *et al.*[9] reported that *P. amarus* act to stimulate the kidney directly to secrete erythropoietin and to stimulates hematopoiesis. This is thought to be due to the iron content of the plant as does other plants with high iron content.

MATERIALS AND METHOD

Collection and Identification of the Plant

Fresh leaf *Phyllanthus amarus* were collected around Mkar hill behind University of Mkar and were identified by Mr Alfred Ozioko, a taxonomist at the centre for Ethno medicine and drugs development Nsukka Enugu State.

Preparation of Extract

Fresh plants of *Phyllanthus amarus* were collected and air-dried under room temperature for 21 days until constant weight was obtained. The leaves were separated from the whole plant and sieved to remove other particles (Stem, Roots, and Seeds). 40g of *Phyllanthus amarus* leaf was macerated in 800ml of water, shaken for 10 minutes and allowed to stay for 72 hours at room temperature to achieve maximum extraction [10]. The extract was sieved with a filter cloth and the juice was filtered using cotton wool and later dried in oven at 35^{o} C. The dried filtrate was used to run column chromatography using silica gel (MESH200) for which 100% ethyl acetate fraction was obtained (100E). The dried fraction was later reconstituted in warm distilled water to the required dosage for administration.

Experimental animals

Thirty albino rats (males) weighing between 120-160g were obtained from animal holding unit, University of Mkar, Mkar Benue State Nigeria. They were allowed to acclimatize for one week, after which they were reweighed and kept in well ventilated laboratory cages with 12 hours' day and night cycles. The rats were maintained on a commercial poultry feed (Vital feeds) and drinking water *ad libitum*. The experiment protocol was followed as approved by Institutional Animal's Ethics Committee (IAEC) and animals care was taken in accordance with the guidelines of European convention for the protection of Vertebrate animals and other scientific purposes ETS-124 [11]. The research was carried out following the experimental design in table 1.

Table: 1 Experimental Design

Group	No of Animal	Extract administration(dose)	
A. Normal control	5	Vital feed/ distil H_2O	
B. Negative control	5	Arsenic administration for 10days	
Treatment Groups			
A. Pretreatment	5	C1 low dose (100mg/kg)	Extract administer for 10days after with Arsenic for 10days
	5	C2 high dose (300mg/kg)	
A. Post-treatment	5	D1 low dose (100mg/kg)	Arsenic administer for 10days after with extract for 10days
	5	D2 high dose (300mg/kg)	

Determination of LD_{50}

This was done according to the method of Lorke [12]. 30 Wistar rats weighing between (120g-170g) of both sexes were divided into 7 groups of 3 animals each and the ethyl acetate fraction of *P.amarus* leaf was administered with varying doses of 100,300, 500,1000, 2000, 3000and 5000mg/kg bw to determine LD_{50}using the formula; $LD_{50} = \sqrt{(D_0 \times D_{100})}$. Where D_0=Highest dose that gave no mortality, D_{100}= lowest dose that produced mortality.

Hematological assays

Hematological analysis of the blood samples was performed using an automated hematology analyzer (2800 Hematology Auto-Analyzer) as prescribed by Ode, et al.[13]. Parameters evaluated included white blood cell (WBC), red blood cell (RBC), hemoglobin concentration (Hg), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT).

Biochemical Analysis

Serum samples were collected from different groups and analyzed for Total Bilirubin (TB), Total Protein(TP), Albumin Blood test (ALB) and Globulin test following standard procedure, using packed kits made by Agappe (Switzer land) Randox(UK).

Collection of Serum.

The animals were sacrificed at NVRI Vom Jos, plateau State at the biochemistry lab. Blood was collected Viajugular vein into a sterile container centrifuge tube and allowed to clot for 2 hours before centrifuging at 3000 rpm for 15 minutes using uniscope laboratory centrifuge.

Statistical Analysis

Data obtained were expressed as mean±SD using one way analysis of variance (ANOVA).The differences among means were calculated at the level of P≤0.05 using Turkey HSD contrast analysis. The values at P≤0.05 were regarded as significant as compared with appropriate control.

RESULTS

The results of ethyl acetate leaf fraction of *Phyllanthus amarus* on LD_{50}, biochemical and hematological parameters of Albino rats are presented in tables 1,2 and 3 respectively.

Table: 1 Determination of LD_{50} of 100% Ethyl acetate fraction of *P. amarus* leaf

Dosage (mg/Kg/bw)	Observed time and Number of deaths	
	Acute toxicity 24 hrs period	Sub-acute toxicity testing 10 days period
5000	0/3	3/3 death
3000	0/3	3/3 death
2000	0/3	3/3 death
1000	0/3	3/3 death
500	0/3	0/3
300	0/3	0/3
100	0/3	0/3

The formula: $LD_{50} = \sqrt{(D_0 \times D_{100})}$ *was used for computation.*

Where D_0*=Highest dose that gave no mortality,* D_{100}*=lowest dose that produced mortality.*

There was no death recorded for the 24 hours administration of the plant fraction and no apparent signs of toxicity. However, a continuous administration, 100 per cent death was observed on the 10th day in groups administered with 1000mg and above as shown in table 1.

Table: 2 Effect of Ethyl Acetate Fraction of *Phyllanthus amarus* Leaf on Hematological *Indices* in Arsenic Administered Wister Rat.

GROUP	WBC10^9/L	RBC10^{12}/L	HGBg/L	HCT%	MCV fL	MCHpg	MCHCg/L	RDW%	PLT10^9/L	MPVfL	PDW	PCT%
A. Positive control	13.59 ±0.10	9.40±0.02	153.58 ±3.55	53.47 ±0.87	57.60 ±1.34	16.58 ±0.25	294.60 ±6.31	15.36 ±0.32	708.80 ±28.37	7.43 ±0.56	34.22 ±0.50	0.55 ±0.02
B. Negative control	7.53±2.16[a]	7.28±0.16[a]	117.80 ±6.06[a]	37.48 ±5.77[a]	56.20 ±2.59*	16.24 ±0.63*	284.60 ±3.78*	15.18 ±0.75*	366.62 ±16.68[a]	5.88 ±1.10[a]	27.08 ±3.53[a]	0.80 ±0.77*
C_1. Pre-treatment Low dose	6.40±0.90	8.30±0.19[b]	143.60 ±2.19[b]	50.48 ±0.15[b]	56.80 ±0.84*	17.34 ±1.01*	288.40 ±2.19*	15.36 ±0.21*	369.80 ±57.40	7.15 ±0.42[b]	32.48 ±0.55[b]	0.25 ±0.27*
C_2. Pre-treatment High dose	10.08±2.45[c]	8.17±0.42	134.60 ±6.91[b]	46.01 ±2.10[b]	56.00 ±1.58*	16.40 ±0.26*	291.00 ±4.64*	16.60 ±1.31*	434.00 ±30.27	6.74 ±0.90	33.86 ±1.89[b]	0.33 ±0.50*
D_1. Post-treatment Low dose	8.48±1.11	8.73±0.08[b]	141.60 ±6.01[b]	49.29 ±1.20[b]	56.00 ±1.58*	16.66 ±1.03*	292.00 ±5.15*	15.56 ±0.18*	531.60 ±59.10[b y]	6.66± 0.34	31.10 ±0.84	0.37 ±0.45*
D_2. Post-treatment High dose	6.41±2.15	7.60±1.09	128.00 ±16.00	43.29 ±5.22	58.20 ±1.30*	17.24 ±0.89*	295.40 ±9.06*	16.10 ±0.92*	449.40 ±56.11[b y]	7.00 ±1.07[b]	33.94 ±4.24[b]	0.31 ±0.50*

Results are expressed in Mean ± SD. (N=5). [a]*Significant at P< 0.05, compared to Positive control.* [b]*Significant compared to Negative control.* [c]*Significant compared to Pre-treatment Low dose.* [y]*Significant compared to Pre-treatment Low and High dose. *not significant compared to normal and negative controls.*

From the result in table 2, there was an evidence of arsenic poisoning indicated by the significant decrease in the levels of some hematological indices like WBC, RBC, HGB, HCT, PLT, MPV, PDW and a significant increased TB in the negative control group administered with sodium arsenate compared to the positive control group without arsenate. However, following administration for ten days before and after poisoning with ethyl acetate fraction of *Phyllanthus amarus* leaf as indicated in table I, significant increase in RBC, HGB, HCT, PLT, PDW (WBC, MPV but not statistically significant) were observed with decreased TB at various doses.

Table3. Effect of Ethyl Acetate Fraction of *Phyllanthus amarus* Leaf on Biochemical Parameters in Arsenic Administered Wister Rat

GROUP	Total Bilirubin mg/dl	Albumin g/l	Glubulin g/l	Total Protein g/l
A. Positive control	1.52±0.17	36.17±1.32	44.22±4.12	80.39±6.20
B. Negative control	2.82±0.19 [a]	33.70±1.11*	48.23±2.01*	81.93±4.20*
C_1. Pre-treatment Low dose	1.74±0.11 [b]	32.10±1.10*	37.04±1.04 [b]	69.14±2.13 [b]
C_2. Pre-treatment High dose	1.59±0.58 [b]	30.56±6.87*	52.45±2.32*	83.01±3.65*
D_1. Post-treatment Low dose	1.25±0.20 [b]	35.80±1.69*	41.38±0.24*	77.18±2.85*
D_2. Post-treatment High dose	1.80±0.74 [b]	32.46±5.02*	46.72±0.92*	79.23±6.26*

*Results are expressed in Mean ± SD. (N=5). [a]Significant at P< 0.05, compared to Positive control. [b]Significant compared to Negative control. [c]Significant compared to Pre-treatment Low dose. [y]Significant compared to Pre-treatment Low and High dose. *not significant compared to normal and negative controls.*

DISCUSSION

Exposure to environmental pollutants constitutes a major threat to animal and human survival in the ever increasing industrialized world. Arsenic contamination of drinking water from various sources has been reported in many parts of the world including developed and developing countries [14]. The assessment of hematological indices provides important information on the alterations that affect the physiology and hematopoietic of blood in disease states or exposures to toxic pollutants [2, 15], in this case arsenate poisoning. Analysis of blood indices is believed to be relevant in risk evaluation and response to therapy as changes in the hematological system have high predictive value for humans when the data are translated from animal studies [1] simply, a significant clinical rise or decline in these indices (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PCT) could suggest excess or suppressed hemopoiesis [15, 16]. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), are related to individual red blood count (RBCs) while the Hemoglobin (HGB), Red Blood Cells (RBC) are relate to the total population of red blood cells in the blood. The significant decreased in RBC, HGB, HCT, PLT, MPV and PDW indicates alterations that affect the physiology and hematopoietic of blood by the arsenate administered and with normal levels of MCV and MCH, this suggest normocytic anemia[17].Furthermore, decrease level of HGB observed in the negative control could implies that arsenic might have either affected the incorporation of hemoglobin in the red blood cells or the morphology and osmotic fragility in the red blood cells produced and this can affect the oxygen carrying capacity of the blood, although with MCV and RDW both normal, the RBCs are likely to be about the same normal size. According to Olufunkeet al. [14], arsenic is known to cause inhibition of aminolevulinic acid dehydratase activity, thereby altering the hem synthesis pathway.

Significant falls in Platelets count with low MPV and irregular morphology as indicated by PDW infer that the bone marrow isn't producing enough new platelets. This may be due to a disorder affecting production by the bone marrow, which may result in low clotting capacity of the blood. And this is not caused by infection from bacteria as procalcitonin (PCT) in all the groups showed no significant difference but the effect on the physiology of the blood was that of the arsenic poison. The result also showed increased bilirubin. Liver uses bilirubin to produce bile, an increase in bilirubin level above normal is an indication of liver cell injury[18]. Thus according to Singh et al.[18], significant increase in bilirubin with or without an increase in ALT indicates cholestasis. WBCs also drop significantly, suggesting susceptibility to opportunistic infection

However, these effects were significantly (P<0.05) ameliorated in the treatment groups, as the levels of these parameters were reversed. This is because *P. amarus* is rich in phenolics [19]. Phenolics excite their mechanism of therapeutic effects either by ways of antioxidant activities as free radical scavengers or chelators of metal ions thus reducing their pro-oxidant activity [20] or by modulation/regulation or inhibition of cell signaling pathways, or by their effect on cholesterol synthesis or their reduction of platelet aggregation and/ or their effect on blood pressure and hormone metabolism [21, 22, 23].It has been previously suggested by Ita, et al.[9], that bioactive components of *P. amarus* act to stimulate the kidney directly to secrete Erythropoietindue to its iron content) and stimulates hematopoiesis.

CONCLUSION

Assessing hematological indices is crucial to probe the therapeutic or order wise the toxicity of medicinal plants. A rise or decline in hematological variables could suggest excess or suppressed hemopoiesis. Thus the research showed agitation on hemopoiesis by arsenate poison which was significantly ameliorated by ethyl acetate fraction of *P. amarus* leaf administration.

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Competing Interests

Authors have declared that no competing interests exist.

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