



## **Effect of Aqueous Leaf Extract of *Acalypha wilkesiana* on Hematological Parameters in Male Wistar Albino Rats**

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### **Authors' contributions**

All authors read and approved the final manuscript. Author OMI designed the study, performed the statistical analysis and wrote the protocol and the first draft of the manuscript. Author OE managed the laboratory analysis of the study. Author EBO managed the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aims:** The aim of this study is to evaluate the effect of aqueous leaf extract of *Acalypha wilkesiana* on hematological parameters in male wistar rats.

**Study Design:** *In vivo*.

**Place and Duration of Study:** Department of Biochemistry, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma Nigeria, between August 2011 and October 2011.

**Methodology:** Thirty two male wistar rats of average body weights 167.50g were grouped into four (I-IV), of eight rats each. Group I received distilled water (control), while constituted doses of 2500, 5000 and 10000 mg/kg body weight of the extract were administered once daily for 14 days to animals in group II, III and IV respectively. The effect of administration of this extract on hematological parameters was evaluated.

**Results:** Results showed that the extract did not exhibit any significant effect ( $P > 0.05$ ) on packed cell volume, hemoglobin, red blood cell count, white blood cell, neutrophil, lymphocytes, platelets, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and erythrocyte sedimentation rate at all the administered doses of the extract. There was a significant reduction in mean corpuscular volume at all doses of the

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administered extract when compared with the control.

**Conclusion:** The extract may be considered relatively hematotoxic at a dose of 2500 mg/kg due to its potentials to cause the formation of microcytic RBC's.

*Keywords: Packed cell volume; hemoglobin; red blood cell count; white blood cell; neutrophil.*

## 1. INTRODUCTION

The use of medicinal plants has been part of human culture in Nigeria, and a large percentage of the people depend on herbal medicines because the international commercial medicines are becoming increasingly expensive and out of reach [1]. *Acalypha wilkesiana* is one of several medicinal plants used in Nigeria. *Acalypha wilkesiana* belongs to the family *Euphorbiaceae*. It is propagated by stem cuttings at any time of the year. Under ideal conditions, it grows as a spreading evergreen shrub with upright branches that tend to originate near the base and can get up to 3.1 m tall with a similar spread. It has leaflets (12.7-20.3 cm long) that are alternate, elliptic to oval, serrate and multicolored and small inconspicuous flowers (10.2-20.3 cm) that hangs in catkin-like racemes beneath the foliage [2].

*A. wilkesiana* has antihyperglycemic [2] and antihypertension properties [3]. Aqueous leaf extract of *A. wilkesiana* is traditionally used to treat neonatal jaundice in western part of Nigeria on short-term basis. However, due to the current publicities on the alleged efficacies of *A. wilkesiana* in the treatment and management of various ailments, there is an increase in the traditional uses of the plant to the extent that the leaf extract of *A. wilkesiana* is consumed in large quantities. Hence, there is a need for a continuous scientific investigation of the toxic implications or safety of the leaf extract of *A. wilkesiana*. Previous work on toxicity testing of this aqueous leaf extract was done by Iniaghe et al. [4]. Results showed that it had hyponatremic properties with no adverse effect on the liver.

The objective of this study, therefore, was to investigate the effect of aqueous leaf extract *A. wilkesiana* on the hematological parameters in male wistar albino rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials for Analysis

Fresh mature *A. wilkesiana* leaves were collected from the natural habitat in August within Ambrose Alli University, Ekpoma, Edo State, Nigeria and the authentication of the plant has been obtained from same. The leaf were picked, washed in distilled water and spread in the sun. The dried leaf was ground to powder using an electric grinder. The leaf powder (1 kg) was suspended in four liters of distilled water and boiled at 100°C for one hour. The suspension was allowed to attain room temperature and filtered, using Whatman No 1 filter paper. The filtrate was evaporated to dryness at 50°C using a water bath and the percentage yield of the extract was 8.54% (w/v). The pasty residue was used to prepare a standardized solution of the leaf extract in calculated amount of distilled water with standardized concentration of 2.96 g/ml. The standardized solution (the stock solution) was stored air tight in plastic bottles and kept frozen.

### **2.1.1 Laboratory Animals**

Thirty two male Wistar rats weighing 140-180g were obtained from the Animal Care Facility, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The rats were fed with rat pellet (product of Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria). The Animal Ethics Committee of Department of Biochemistry, Faculty of Natural Sciences of Ambrose Alli University, Ekpoma, Nigeria approved all experimental protocols.

### **2.2 Experimental Animals and Procedure**

The Thirty two male wistar rats were randomly grouped into four, comprising of eight rats per group. The rats were housed in cages made of wooden frames and metal netting, and were fed with rat pellet and tap water *ad libitum* with 12-hours light/dark cycle. The cages were cleaned every morning and disinfected at intervals of 3 days. The rats were allowed to acclimatize for 10 days before extract administration was commenced. Calculated amount of aqueous leaf extracts of *A. wilkesiana* were constituted in distilled water from the stock solution of 2.96g/ml to give doses of 2500, 5000 and 10000 mg/kg body weight and administered to the various groups as illustrated:

- Group I : control, received 1.0 ml distilled water
- Group II : received 2500 mg/kg body weight of the extract
- Group III : received 5000 mg/kg body weight of the extract
- Group IV : received 10000 mg/kg body weight of the extract

Administration of aqueous leaf extract of *A. wilkesiana* was performed orally once daily between 9:30 am  $\pm$  30 minutes, using metal cannula attached to a 2ml syringe. Administration lasted for 14 days, after which the rats were fasted for 12 hours, and were sacrificed by anesthesia using chloroform. Blood was collected by cardiac puncture and the blood placed in EDTA bottles for hematological analysis.

#### **2.2.1 Gross necropsy and observation**

During the period of experimentation, all the animals used were subjected to a detailed gross necropsy that included careful examination of the external surface of the body, all orifices and cranial, thoracic and abdominal cavities. Behavioral changes, depression, salivation, diarrhea, muscular weakness and sedation were also observed.

#### **2.2.2 Hematological analysis**

The effects of the extract on packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC), neutrophil, lymphocytes, platelets, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and erythrocyte sedimentation rate (ESR) were analyzed using an automated analyzer (SYSMEX K-21N: SYSMEX CORPORATION, JAPAN).

### **2.3 Statistical Analysis**

The results were expressed as mean  $\pm$  standard deviation (SD) and were subjected to one way analysis of variance (ANOVA), using statistical package for social sciences (SPSS-15) at 95% level of confidence. The least significant difference (LSD) was performed for the pair-

wise mean comparisons, to determine the significant treatment dose at 95% level of confidence. Values were considered statistically significant at ( $P < 0.05$ ) and denoted by different alphabets.

### 3. RESULTS AND DISCUSSION

Toxicity studies of herbal extract in animals are commonly used to assess potential health risk in humans, caused by intrinsic adverse effects of chemical compounds or plant extracts [5]. The deleterious effects of these extracts may be accompanied or preceded by clinical signs of toxicity such as salivation, loss of hair, changes in animal eye color, decreased respiratory rate and motor activity. The various hematological parameters investigated in this study are useful indices that can be employed to assess the toxic potentials of plant extracts/botanicals in living systems [6]. Such toxicity testing is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity, when data are translated from animal studies [7] at an administered dose of 10,000 mg/kg body weight of aqueous leaf extract of *A. wilkesiana*, all rats displayed normal behavioral, neurological and autonomic profiles. No mortality or morbidity was observed in the rats at 10,000mg/kg body weight of the extract. This is an indication that the extract may have been well tolerated by the rats.

Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal [8]. It can also be used to explain blood relating functions of chemical compounds/plant extract [9]. Hematological parameters provide information regarding the status of bone marrow activity and hemolysis. The various hematological parameters investigated in this study as shown in Table 1 are useful indices that can be employed to assess the toxic potentials of plant extracts/botanicals in living systems [10]. In the present study, the administration of aqueous leaf extract *Acalypha wilkesiana* did not exhibit any significant effects on the hematological parameters investigated, except the MCV (Table 1). There was a significant reduction in mean corpuscular volume at all doses of the administered extract when compared with the control.

MCH, MCHC and MCV relates to individual red blood cells while Hb, RBC and PCV are associated with the total population of red blood cells [11]. The non-significant effect of the extract on RBCs might be an indication that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) was not altered. Reduction in platelets count in experimental animals has been reported to indicate adverse effect on the oxygen-carrying capacity of the blood as well as thrombopoietin [12]. Results from this study show that the platelet count was unaltered signifying that the oxygen carrying capacity of the blood was unaffected when this extracted was administered at a dose of 10,000mg/kg to the male Wistar rats.

The MCV is an index of the size of the RBCs. When the MCV is below normal, the RBCs will be smaller than normal and are described as microcytic. When the MCV is elevated, the RBCs will be larger than normal and are termed macrocytic. RBCs of normal size are termed normocytic [13]. These size categories are used to classify anemias.

**Table 1. Effect of aqueous leaf extract of *Acalypha wilkesiana* on hematological parameters in male wistar rats**

Parameters	Control	2500mg/kg	5000mg/kg	10,000mg/kg
PCV (%)	43.4 ± 4.70	41.0 ± 2.11 <sup>a</sup>	41.2 ± 3.35 <sup>a</sup>	41.3 ± 1.45 <sup>a</sup>
RBC ( X10 <sup>6</sup> /μL)	6.83 ± 0.83	6.99 ± 0.29 <sup>a</sup>	8.57 ± 2.11 <sup>a</sup>	8.65 ± 2.97 <sup>a</sup>
Hb (g/dl)	12.55 ± 1.42	12.78 ± 0.46 <sup>a</sup>	8.83 ± 3.57 <sup>a</sup>	11.29 ± 2.83 <sup>a</sup>
WBC (x10 <sup>4</sup> /mm <sup>3</sup> )	1.18 ± 0.20	1.25 ± .044 <sup>a</sup>	1.20 ± 0.13 <sup>a</sup>	0.93 ± 0.20 <sup>a</sup>
NEUTROPHIL (%)	23.58 ± 3.60	31.83 ± 22.98 <sup>a</sup>	30.37 ± 11.45 <sup>a</sup>	38.13 ± 20.84 <sup>a</sup>
LYMPHOCYTE (%)	75.50 ± 2.05	61.45 ± 15.31 <sup>a</sup>	66.07 ± 9.28 <sup>a</sup>	54.95 ± 20.84 <sup>a</sup>
PLATELETS(x10 <sup>5</sup> /mm <sup>3</sup> )	5.94 ± 1.65	7.56 ± 0.20 <sup>a</sup>	7.34 ± 2.20 <sup>a</sup>	6.58 ± 1.39 <sup>a</sup>
MCV (fl)	63.68 ± 2.82	58.78 ± 1.03 <sup>b</sup>	59.84 ± 1.96 <sup>b</sup>	57.38 ± 2.52 <sup>b</sup>
MCH (pg)	18.38 ± 0.81	18.53 ± 0.53 <sup>a</sup>	18.58 ± 0.40 <sup>a</sup>	17.73 ± 1.24 <sup>a</sup>
MCHC (g/dl)	28.65 ± 2.08	31.48 ± 1.45 <sup>a</sup>	30.65 ± 0.77 <sup>a</sup>	30.93 ± 1.87 <sup>a</sup>
ESR (mm/hr)	1.33 ± 0.52	1.00 ± 0.00 <sup>a</sup>	1.17 ± 0.41 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>

(PCV, Packed cell volume; RBC, Red blood cell; Hb, hemoglobin; WBC, White blood cell; MCV, mean corpuscular volume, MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration, ESR, erythrocyte sedimentation rate). Results are expressed as Mean ± SD, n=8. a = values not significantly different from control ( $P > 0.05$ ); b = values significantly different from control ( $P < 0.05$ ).

Failure to produce hemoglobin results in cells smaller than normal cells. This occurs in many diseases, including iron deficiency anemia, thalassemia (an inherited disease in which globin chain production is deficient), and anemias associated with chronic infection or disease. Iron is an essential component of many enzymes in cells and is also part of the heme group in hemoglobin (which consists of a porphyrin ring containing iron). Much of the body's iron stores are within red blood cells where iron is critical for hemoglobin synthesis. Iron deficiency could be due to inadequate intake or absorption of iron, excessive loss with external hemorrhage, or interference with iron metabolism [13,14,15]. In this study, there was a significant reduction in mean corpuscular volume at all doses of the administered extract when compared with the control suggesting that this extract may be interfering with iron uptake into hemoglobin. The decrease in MCV observed in this study is contrary to that observed when this same extract was administered to alloxan induced diabetic rats. The extract (100mg/kg - 300mg/kg) increased significantly the level of MCV when compared with the test control group [16]. The medicinal property of this extract is likely more visible at lower doses of the extract. The trend obtained in this study is similar to that obtained when *Sorghum bicolor* was to treat Albino rats [17]. The MCV values increased from the extract dose of 200mg/kg to 400mg/kg body weight. However at 1,600mg/kg the MCV value was significantly lower than the value obtained at the dose of 200mg/kg body weight. 60mg/kg of aqueous leaf extract of *Acalypha racemosa* (a different specie) has been shown to be hepatoprotective against carbon tetrachloride induced hepatotoxicity [18]. Toxicity studies on higher doses of *A.wilkesiana* leaf extract in the range of 2500mg/kg to 10,000mg/kg did not show any sign of hepatotoxicity or nephrotoxicity in normal male wistar rats [19].

#### 4. CONCLUSION

Though the aqueous leaf extract of *Acalypha wilkesiana* did not cause either mortality or obvious morphological toxic effect, it may be considered relatively hematotoxic at a dose of 2500 mg/kg due to its potentials to cause the formation of microcytic RBC's.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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