Effect Of Aqueous Leaf Extract Of *Acalypha Wilkesiana* Muell-Arg (Jacob's Coat,Copper Leaf) Leaves On Leucocyte Count (Wbc) In Mice After Subacute Administration

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ABSTRACT: The dried and pulverized leaves of *Acalypha wilkesiana* was reflux with distilled water. Studies were then undertaken on the effect of the aqueous extract on leucocytic count (WBC) and differential leucocyte count (DLC) on Mice graded doses (800mg/kg,1000mg/kg body weight) of the extract were administered orally to different groups of mice daily for 21 days and had a beneficial effects on the WBC and DLC. There was a significant ($p<0.05$) increase in leucocyte count when compared to control in all the days (7, 14 and 21). Differential leucocyte count also indicated a significant ($p<0.05$) increase while Lymphocyte indicated a significant ($p<0.05$) decrease.The prolong oral administration of the extract under the condition of this study shows that the extract may be toxic at higher doses. Nevertheless,the extract appeared to be more beneficial at lower doses and significantly ($p<0.05$) improves WBC and DLC values and this effect has potential application in stimulation of the immune pathway. This may seem to explain for its use as an antifungal and antibacterial agent in African traditional medicine.

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INTRODUCTION

The plant *Acalypha wilkesiana* Muell-Arg (Jacob's coat, Copper leaf, fire dragon) is one of the most widely known and utilized of the family Euphorbiacaece. The genus comprises about 570 species (Riley, 1963) with a large proportion as weeds while others are ornamental plants. They are found world wide mostly around the tropics of Africa, America and Asia. The leaves measure 4-8 Inch as heart-shaped with a combination of colours like green, purple, yellow, orange, pink or white depending on cultivation. The dense, much branched growth habit creates a full shape but plants occasionally need chopping to maintain a neat appearance. Its upright growth can reach 10-15 feet in height making it well suited to be used as accent in mixed shrubbery borders (Edward, 2011). Its growth rate being fast, it is an all year round plant as long as it is provided with water and sunlight. It can grow in partly illuminated environment. Although copper leaf grows easily in sun, frost free locations, the plant branches less in partial shade. It looks its best when provided with regular watering during drought and will grow on a wide variety of garden soils, easily propagated by air – layers on cutting (Edward, 2011).

The important role of traditional medicine in the health care delivery system of many developing countries cannot be over emphasized. In addition traditional system is now a subject of global importance (UNESCO,1994). Traditional Medicine is the first choice of health treatment for at least 80% of Africans who suffer from various forms of ailment (Elujoba.*et al*., 2005).

The leaves of *Acalypha wilkesiana* are eaten as vegetables in the management of hypertention (Ikewuchi. *et al*., 2008). The expressed juice or boiled decoction is used for the treatment of gastrointestinal disorders and fungal infections (Oliver, 1959). Therefore this study is an attempt to investigate the leucocytosis response of *Acalypha wilkesiana* leaves in mice after sub acute administration.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh leaves and stem of *Acalypha wilkesiana* were collected in the month of June 2008 from University of Maiduguri Staff Quarters. The plant was identified and authenticated by a botanist from the Department of Forestry, University of Makurdi, Benue State.

Preparation of extract

Fresh leaves of Acalypha wilkesiana was air dried and pulverized by grinding using mortar and pestle. 200g of ground leaves was exhaustively extracted with distilled water using reflux (Trease and Evans, 1989). The extract yield was 22.9% $^{w}/_{w}$. The resultant extract was concentrated in vacuo, properly labeled and stored in a specimen bottle in the refrigerator at 4^0C (Trease and Evans,1989) until when required.

Experimental Animals

A total of 100 adult mice were used for the experiments. They were obtained from the animal house of the Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. They were housed in clean plastic cages in Veterinary Physiology, Pharmacology and Biochemistry Laboratory of the University of Maiduguri for two weeks for acclimatization, before the commencement of the experiments. They were fed with growers' mash (Vital feeds^R Nig. Ltd. Jos) and clean water *ad libitum*. The animals were handled according to the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1985) as certified by Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

Sub-acute Toxicity Studies

One hundred (100) adult mice were selected and divided into four groups of 25 mice each. Group A was the control. Groups B, C and D were treated orally with 800 mg/kg, 1000 mg/kg and 1200 mg/kg of aqueous extract of *A. wilkesiana,* respectively for a period of 3 weeks (21days) while group A received distilled water only by the same route for the same period of time. The animals were bled weekly and the blood used for the determination of white blood cell count (WBC) and differential leucocyte counts (DLC) using standard procedure (Coles, 1986; Schlam, *et al*., 1975).

Statistical Analysis

All values were expressed as Mean ± Standard Deviation (S.D). Analysis of variance (ANOVA) was used to analyze the extent of variation among groups and P values equal to or less than 0.05($P \leq 0.05$) were considered significant. The computer statistical soft ware Graph Pad Instat (2003) was used to analyze the data.

RESULTS

Effect of aqueous extract of *Acalypha wilkesiana* leaves on White Blood Cell (WBC) Count in Mice

The effect of the aqueous leaf extract of *A.wilkesiana* on white blood cell (WBC) count in mice indicated that all the doses provided significant ($p<0.05$) increase in white blood cell (WBC) when compared to the control group. Following the withdrawal of the extract, the white blood cell count of the mice at 28th day and 35th day (7th day and 14th day post extract withdrawal) were significantly ($p<0.05$) higher than the control.

Table 1: Effect of prolonged oral administration of aqueous extract of *Acalypha wilkesiana* leaves on White Blood Cell (WBC) x10^3/L Count concentration

	Extract Treatment	Treatment Day			Days after withdrawal of treatment	
		7	14	21	7	21
	Dose (mg/kg)	Mean± SD				
White Blood Cell (WBC)	Control (Distilled water)	6864.2± 023.90^a	8780.6±390.2^a	5800.0±509.9^a	8080.0±238.7^a	8322.8±190.8^a
	800	7320.0±363.3^a	97324.0±202.1^b	12240.0±2639.7^b	9900.0±1086.3^b	10120.0±1485.6^b
	1000	10701.6±1749.4^b	6698.6±5223.0^a	8443.0±832.9^b	10380.0±867.2^b	9360.0±594.1^b
	1200	7660.2±1199.0^a	10590.0±900.3^a	6916.0±5377.9^b	8660.0±497.9^b	10080.0±356.4^b

$^{a, b,}$ means with different superscript are significantly ($P<0.05$) different**.** n=5

Effect of aqueous extract of *Acalypha wilkesiana* leaves on Relative Differential Leucocytes Count (DLC) or Percent Cell Type in mice.

Lymphocyte count indicated a significant ($p<0.05$) decrease in all the days compared to their control mice. Neutrophils showed significant ($p<0.05$) increase in all the days except for day 14 of withdrawal which showed no significant ($p>0.05$) difference as compared to their control.

Day 7 of treatment showed no significant ($p>0.05$) difference in eosinophils level in mice with day 14 of withdrawal but the rest of the days showed significant ($p<0.05$) increase as compared to control while bosophils days 14 and 21 of treatment showed

a significant ($p<0.05$) increase when compared to the control but withdrawal days showed no significant ($p>0.05$) difference.

Day 7 of treatment indicated no significant ($p>0.05$) difference in monocyte level treated mice although subsequent days, 14 and 21 of treatment showed significant ($p<0.05$) increase together with day 7 of withdrawal with the group that has taken the highest dose while day 14 of withdrawal indicated no significant ($p>0.05$) difference when compared to control.

Relative Number of DLC or Percentage Cell Type $= \frac{Number\ of\ that\ cell\ type}{Total\ WBC\ count}$

Table 2: Effect of prolonged oral administration of aqueous extract of *Acalypha wilkesiana* leaves on Relative Differential Leucocyte Count (DLC) concentration or Percent Cell Type

	Extract Treatment	Treatment Days		Days after withdrawal of treatment		
		7	14	21	7	14
$\times 10^9$/L	Dose (mg/kg)	Mean± SD				
Lymphocyte	Control(Distilled water)	69.0±07.10[a]	67.8 ±0.84[a]	68.8 ±0.84[a]	67.8 ±1.64[a]	68.4 ±0.55[a]
	800	64.6 ±11.40[b]	55.6 ±1.82[b]	53.8 ±3.11[b]	63.4 ±2.30[b]	66.8 ±1.92[a]
	1000	64.2 ±1.30[b]	52.4 ±1.67[b]	45.2 ±0.84[b]	60.4 ±1.67[b]	65.8 ±1.09[b]
	1200	62.6 ±0.89[b]	47.6 ±2.70[b]	42.4 ±1.52[b]	57.8 ±1.30[b]	64.6 ±1.52[b]
Neutrophils	Control (Distilled water)	24.4±0.89[a]	25.0±1.41[a]	23.0±1.58[a]	23.2±1.30[a]	24.6±1.52[a]
	800	28.2±0.84[b]	31.0±1.00[b]	33.2±1.64[b]	26.6±1.67[b]	25.4±1.14[a]
	1000	28.2±0.84[b]	32.2±1.30[b]	39.2±0.84[b]	29.4±1.14[b]	26.0±0.71[a]
	1200	29.0±1.00[b]	35.0±2.35[b]	40.0±1.58[b]	29.4±0.89[b]	26.8±1.30[a]
Eosinophils	Control (Distilled water)	4.6±0.55[a]	4.8±0.84[a]	5.0±0.71[a]	4.8±0.84[a]	4.6±0.55[a]
	800	4.4±0.55[a]	9.0±1.22[b]	10.0±0.71[b]	6.4±0.55[b]	5.2±0.84[a]
	1000	5.0±0.71[a]	9.8±0.84[b]	9.4±1.14[b]	6.8±0.45[b]	5.4±0.55[a]
	1200	5.4±0.55[a]	10.8±0.84[b]	10.6±0.89[b]	8.2±0.84[b]	5.4±0.55[a]
Basophils	Control (Distilled water)	1.0±0.71[a]	1.4±0.55[a]	1.8±0.45[a]	1.6±0.55[a]	1.4±0.55[a]
	800	1.4±0.55[a]	2.6±0.55[b]	2.8±0.45[b]	2.0±0.00[a]	1.4±0.55[a]
	1000	1.4±0.55[a]	2.8±0.84[b]	3.0±0.71[b]	2.0±0.00[a]	1.4±0.55[a]
	1200	1.6±0.55[a]	3.6±0.89[b]	4.0±0.71[b]	2.2±0.45[a]	1.8±0.45[a]
Monocytes	Control (Distilled water)	1.2±0.45[a]	1.4±0.55[a]	1.4±0.55[a]	1.8±0.45[a]	1.6±0.55[a]
	800	1.2±0.45[a]	2.2±0.45[b]	2.2±0.45[b]	1.6±0.55[a]	1.2±0.45[a]
	1000	1.2±0.45[a]	2.8±0.84[b]	3.2±0.45[b]	1.4±0.55[a]	1.4±0.55[a]
	1200	1.6±0.55[a]	3.0±0.71[b]	3.0±0.71[b]	2.4±0.55[b]	1.4±0.55[a]

[a, b,] means with different superscript are significantly ($P<0.05$) different.
n=5

DISCUSSION

The significant increase in WBC values may possibly mean stimulation of the immune pathways. Schlam *et al.,* (1975), reported that persistent antigen load in the body would result in increased WBC count. Also there is a significant ($p<0.05$) increase in differential leucocyte count (DLC) indicating that the body was probably reacting to an infection or antigen and thereby stimulation of the immune system. Kashinath, (1990) and Schalm et al.,(1975) reported that biological compounds have been reported to stimulate the immune functions and the persistence of antigenic load in the body results in the development of leucocytosis at the site of injury which improves wound healing. Lymphocyte count in this study shows significant ($p<0.05$) decrease when compared to the control. Lymphopaenia result from changes in lymphocyte kinetics stimulated by acute inflammatory mediators that reduce the circulating lymphocyte pool such as increase migration and emigration of lymphocytes to inflammed tissues, homing of lymphocytes to lymphnode by increasing

the rate of immigration through the post capillary high endothelial cells reducing the rate of lymphocytes leaving lymphnode via efferent lymphatic vessels (Stockham and Michael, (2002). Lymphopaenia is often found in patients undergoing radiotherapy and chemotherapy (Baker *et al*., 1998). However lymphopaenia can also occur due to increase demand on the system for lymphocytes in both immune and inflammatory response (Mbaya *et al*., 2008).

Tissue injury leads to degranulation of mast cells resulting in histamine release. This elevated histamine level attracts eosinophils from the bone marrow into circulation (Schalm et al., 1975). This could be responsible for the eosinophilia observed in this study. Monocytosis could occur due to acute or chronic diseases as a result of increased production in the marrow since there is no large storage pool of monocytes (Schalm et al., 1975). Basophilia is associated with allergic, parasitic and neoplastic states (Stockham and Michael, 2002).

The increase in immune response could also be due to the phytochemical constituents which reveal the presence of carbohydrates, flavonoids, cardiac glycosides. phlobatannins and alkaloids (Madziga *et al.,* 2010). Carbohydrate and its derivatives play major roles in the working process of immune system (Maton *et al.,* 1993). Flavonoids have been referred to as nature's biological response modifiers because of their ability to modify the body's reaction to allergies, viruses and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (Yamato and Gayor, 2002).

CONCLUSION

In conclusion, the aqueous leaf extract of *Acalypha wilkesiana,* when observed in mice following prolonged oral administration, indicated an increase in WBC and DLC count probably indicating immune stimulation, but there was a decrease in lymphocytic count. Caution should be taken on prolonged administration of this extract especially in high dosage.

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