Research Article

Toxicity study of aqueous extract of *Acalypha wilkesiana* Muell-Arg (Jacob's coat, Copper leaf) leaves on some haematological parameters in mice

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Received: 10 January 2020

Revised: 18 February 2020

Accepted: 24 February 2020

Abstract

Objective: Objective of present study was to evaluate the efficacy and safety of *Acalypha wilkesiana* on some haematological parameters in mice. **Materials and Methods:** The oral acute toxicity (LD₅₀) was studied using the method of Kaber as modified by Aliyu and Nwude. The effects of the aqueous extract of *Acalypha wilkesiana* leaves on some haematological parameters in mice were studied. **Results:** The oral LD₅₀ with 95% confidence interval of the aqueous leaf extract was estimated to be 1420mg/kg, indicating that the extract is not toxic. After prolonged oral treatment of mice with the leaf extract, significant increases in the levels of mean Hb concentration, RBC, and PCV were observed. **Conclusion:** This study suggests that the aqueous leaf extract of *Acalypha wilkesiana* contains some important chemical components which may have therapeutic values and have some beneficial effects on haematological parameters, suggesting that it could be used as an anti anaemic.

Keywords: *Acalypha wilkesiana*, acute toxicity, hematological parameter

Introduction

Acalypha wilkesiana belongs to the family Euphorbiaceae. The common names for this plant include, copper leaf, fire dragon, beef steak plant, Jacobs coat (Riley, 1963), and locally called eda or jiwanen. The plant is found all over the world, especially in tropic of Africa, America and Asia (Ogundaini, 2005). Traditional medicine has been practiced to some degree in all cultures. The traditional medical systems are based on experiments in using plant products in eradication of common ailments (Gupta, 1994). Thus, the World health Organization (WHO) has encouraged research on herbal medicines with a view to developing and incorporating the effective locally available substitutes/herbs remedies into the health care system (Abdulrahman et al. 2005). However, in traditional medicine, there is no proper dosage or quantity of the material given to recipients and the nature and type of active ingredient in the

mixture may not be known. In such practices, the probability of administering toxic doses is high (Musa et al., 2005). Acute toxicity study is important since science requires the validation of drugs by medical practitioners and drug regulatory authorities demand that all potential drugs should pass through a rigorous series of study and scrutiny (Iwu, 1996; Abdulrahman, 2004). Recently some researchers confirmed some of the Folk use (Ogundaini 2005; Adesina et al., 2000) however there is not much study on this plant in the area acute toxicity and hematological parameters. Therefore, this study was design to conduct the acute toxicity studies of the leaf of the plant and to determine the effects of the extract on some haematological parameter in mice.

Materials and Methods

Plant collection and identification

Fresh leaves and stem of *Acalypha wilkesiana* were collected in the month of June 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.

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DOI: <https://doi.org/10.31024/apj.2020.5.1.2>

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Extract preparation

The fresh leaves were air-dried at room temperature (37°C) for two weeks. The dried leaves were then pulverized using a wooden mortar and pestle and then ground into powder form. Two hundred gram (200g) of the powdered form was poured into a round bottom flask and 1 liter (1000mls) of distilled water added after which the mixture was placed on a hot plate and allowed to boil for 1 hr. It was then allowed to cool and then filtered using Whatman filter paper No. 1, which was then evaporated to dryness using oven at 50°C and was later stored at 4°C until used. The concentration of the extract was 100 mg/ml and the yield was 22.85%W/W.

Experimental animals

A total of 105 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[®] Nig. Ltd. Jos) and clean water ad libitum. All experiments on animals were carried out according to the biomedical principles involving animals (CIOMS and ICLAS, 2012).

Acute toxicity study

The arithmetic method of Karber (1931) as modified by Aliu and Nwude (1982) was used to evaluate the oral acute toxicity of the aqueous leaves extract of *Acalypha wilkesiana*.

In this method, twenty five (25) adult mice were selected at random and separated into 5 groups of 5 mice each. Groups were designated A, B, C, D and E. Group A, animals which served as the control were given distilled water while the other four groups (B, C, D and E) were given the aqueous leaf extract of *Acalypha wilkesiana* at different dosages 800, 1000, 12000 and 1600 mg/kg respectively and observed for clinical signs and death over a period of 24 hours.

$$LD_{50} = \text{Dose that caused highest No of death} - \frac{\text{Mean dead} \times \text{dose difference}}{\text{Number of mice in each group}}$$

Sub-acute toxicity studies

Eighty (80) adult female mice were selected and divided into four groups of 20 mice each. Group A was the control group. Groups B, C and D were treated orally with 100 mg/kg, 1200 mg/kg and 1600 mg/kg of aqueous extract of *Acalypha wilkesiana*, respectively for a period of 3 weeks (21 days) while group A, (control) received distilled water only for the same period of time.

Haematological analysis

The estimates of the various haematological parameters were carried out on weekly basis for 3 weeks after the extract administration. At the end of each week, blood samples from 5 mice in each group were collected from the tail vein for determination of haematological parameters such as red blood cell counts (RBC), packed cell volume (PCV), mean haemoglobin concentrations (Hb) by haemocytometry, using standard procedures (Cole, 1974; Schalm et al., 1976; Brown, 1976; Dade and Lewis, 1984).

Statistical analysis

All values were expressed as Mean $\pm$ Standard Deviation (S.D). Analysis of variance (ANOVA) was used to analyze the extent of variations among groups and P values equal to or less than 0.05 ($P \leq 0.05$) were considered significant. The computer statistical software GraphPadInstat (2003) was used to analyze the data.

Results

Acute toxicity study

The oral LD_{50} with 95% confidence limit was 1,420mg/kg. From the pilot study of the acute toxicity study done, 100 mg/ml stock concentration of the aqueous extract did not cause death.

Table 1. Calculation of LD_{50} using the Arithmetic method (Aliu and Nwude, 1982)

S. No.	Dosage	Dose Difference	Dead	Mean Dead	Mean dead X dose difference
1	800	200	0	0.5	100
2	1000	200	1	1	200
3	1200	400	1	1.5	600
4	1600		2		
$LD_{50} = 1420\text{mg/kg}$					Total 900

Effect of the extract on haematological parameters

The effect of prolonged oral administration of aqueous extract of *Acalypha wilkesiana* leaves on Red Blood Cell (RBC), Packed Cell Volume (PCV) and Haemoglobin (Hb) count of treated mice are presented in table 2.

At day 7 treatment with 800mg/kg, RBC values significantly ($p < 0.05$) increased from 745.20 ± 34.40 to 843.20 ± 68.90 , similarly at 100mg/kg the RBC values increases to 951.60 ± 41.60 as well as at dose 1200mg/kg with values of 977.20 ± 27.80 . Day 14 treatment at 800mg/kg indicated a significant ($p < 0.05$) increase from 691.40 ± 26.30 to 1096.00 ± 59.50 , also at 100mg/kg and 1200mg/kg the RBC values increased to 1072.60 ± 75.90 and 1105.40 ± 60.20 respectively when compared to 691.40 ± 26.30 which is the control. Day 21 treatment, RBC values also increased significantly ($p < 0.05$) from 643.20 ± 36.40 to 940.40 ± 46.80 at dose of 800mg/kg, 917.00 ± 28.64 at dose 1000mg/kg and also 886.00 ± 57.81 at dose 1200mg/kg respectively. While day 7 and 14 of extract withdrawal, RBC values returned to non-significant ($p > 0.05$) values when compared to control.

PCV values at day 7 of extract treatment with 800mg/kg indicated a significant ($p < 0.05$) increase, from 45.20 ± 1.30 to 51.80 ± 3.49 , similarly at 1000mg/kg the values increased to 53.40 ± 2.07 and also at 1200mg/kg PCV values increased to 53.00 ± 2.55 when compared to the control.

Day 14 treatment at 800mg/kg, 1000mg/kg and 1200mg/kg indicated a significant ($p < 0.05$) increase from 47.40 ± 1.67 to 54.60 ± 1.67 , 53.60 ± 1.67 and 53.00 ± 3.16 respectively, so also day 21 treatment at 800mg/kg, 1000mg/kg and 1200mg/kg indicated a significant ($p < 0.05$) increase from 47.20 ± 1.48 to 55.40 ± 5.08 , 54.60 ± 3.44 and 58.40 ± 1.14 respectively. Following withdrawal of extract at day 7 and 14, the PCV values returned to normal when compared to control.

Hb values at day 7 of extract treatment with 800mg/kg, 1000mg/kg and 1200mg/kg significant ($p < 0.05$) increased to 11.90 ± 1.65 , 11.70 ± 1.44 and 11.80 ± 1.31 , respectively when compared to the control at 9.90 ± 0.36 . Similarly at day 14 with the same dose of extract Hb values indicated a significant ($p < 0.05$) increase from 12.90 ± 0.82 to 17.50 ± 0.76 , 18.10 ± 0.37 and 18.10 ± 0.40 respectively. Also day 21 treatment at 800mg/kg, 1000mg/kg and 1200mg/kg indicated a significant ($p < 0.05$) increase of 14.94 ± 0.46 , 14.32 ± 0.33 and 14.32 ± 0.33 respectively when compared to control 10.74 ± 0.29 . Following extract withdrawal, the Hb values returned to non-significant ($p > 0.05$) values at days 7 and 14.

Discussion

Previous studies on Phytochemical analysis of the aqueous leaf extract of *Acalypha wilkesiana* revealed high presence of carbohydrates, tannins and Flavonoid, and moderate, presence of phlobatannins, Alkaloid, cardiac glycosides, Terpene and

Table 2. Effect of prolonged oral administration of aqueous extract of *Acalypha wilkesiana* leaves on Red Blood Cells, Haemoglobin Concentration and Packed Cell Volume in mice

Parameters	Extract treatment	Treatment Days			Days after withdrawal of treatment	
		7	14	21	7	21
Red Blood Cell (RBC) $\times 10^6 \text{ mm}^3$	Control (Distilled water)	745.20 ± 34.40^a	691.40 ± 26.30^a	643.20 ± 36.40^a	637.60 ± 41.60^a	634.60 ± 15.40^a
	800 (mg/kg)	843.20 ± 68.90^b	1096.00 ± 59.50^b	940.40 ± 46.80^b	718.80 ± 63.60^a	671.60 ± 24.90^a
	1000 (mg/kg)	951.60 ± 41.60^b	1072.60 ± 75.90^b	917.00 ± 28.64^b	675.40 ± 53.90^a	730.20 ± 23.40^a
	1200 (mg/kg)	977.20 ± 27.80^b	1105.40 ± 60.20^b	886.00 ± 57.81^b	716.00 ± 65.43^a	874.00 ± 74.80^a
Packed Cell Volume. %	Control (Distilled water)	45.20 ± 1.30^a	47.40 ± 1.67^a	47.20 ± 1.48^a	45.40 ± 0.55^a	45.40 ± 0.55^a
	800 (mg/kg)	51.80 ± 3.49^b	54.60 ± 1.67^b	55.40 ± 5.08^b	45.60 ± 1.67^a	44.60 ± 0.55^a
	1000 (mg/kg)	53.40 ± 2.07^b	53.60 ± 1.67^b	54.60 ± 3.44^b	45.40 ± 0.55^a	45.80 ± 0.84^a
	1200 (mg/kg)	53.00 ± 2.55^b	53.00 ± 3.16^b	58.40 ± 1.14^b	46.20 ± 0.45^a	45.20 ± 0.45^a
Haemoglobin g/dl. (Hb)	Control (Distilled water)	9.90 ± 0.36^a	12.90 ± 0.82^a	10.74 ± 0.29^a	10.80 ± 0.31^a	10.80 ± 0.31^a
	800 (mg/kg)	11.90 ± 1.65^a	17.50 ± 0.76^b	14.94 ± 0.46^b	11.78 ± 0.94^a	11.06 ± 0.26^a
	1000 (mg/kg)	11.70 ± 1.44^a	18.10 ± 0.37^b	14.32 ± 0.33^b	11.16 ± 0.22^a	11.28 ± 0.33^a
	1200 (mg/kg)	11.80 ± 1.31^a	18.10 ± 0.40^b	14.92 ± 0.38^b	11.26 ± 0.51^a	11.42 ± 0.23^a

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

steroids. Anthraquinone derivatives were not detected, while the elemental analysis showed the presence Cl^- , Na^+ , K^+ , Ca^{2+} , Fe^{2+} , Mg^{2+} , Zn^{2+} , Cu^{2+} and Mn^{2+} in varying quantities. Cd^{2+} and Pb^{2+} were not found (Madziga et al., 2010).

The aqueous Leaves extract of *Acalypha wilkesiana* administered at the various doses appear to have some beneficial effects on the hematological indices given the observed increases in the values of RBC, Hb and PCV.

The improvement of haematological parameters may be an indication of the possible usefulness of this extract as a haematonic and blood enhancer. According to (Brown, 1976, Jour, 2008), anti anaemic agents tend to stimulate production of RBC and improve the values of Hb and PCV. Presence of Carbohydrate may have contributed to its immune effect since carbohydrate is known to have numerous roles in living things, such as the storage and transport of energy and structural components (cellulose in plants, chitin in animals). In addition carbohydrates and their derivatives play major roles in the working process of the immune system, fertilization pathogenesis, blood clotting and development (Maton et al, 1993., Patil and Muskan, 2009). Furthermore the *Acalypha wilkesiana* contained moderately high iron content; the presence of iron therefore could have contributed to the observed improvement of haematological parameters. The increase in the haematological parameters in this study may also be due to the activities of the chemical constituents of the plant; saponins, as found in the extract, are known to hydrolyze and produce sapogenins which may be steroid or triterpene. Shapiro and Greenfield (1987) reported the stimulatory effects of steroid on bone marrow resulting in increased erythropoietin.

Conclusion

The aqueous leaf extract of *Acalypha wilkesiana* has oral LD_{50} 1,420mg indicating that is not toxic. The leaf of *Acalypha wilkesiana* contains some important organic and chemical components which are pharmacologically active and may be beneficial and have therapeutic values, therefore the aqueous extract of this plant could be of immense medicinal value. The aqueous leaf extract of *Acalypha wilkesiana* increased haematological parameters, thus it appears to have some beneficial effect on haematological parameters of mice (RBC, Hb and PCV) suggesting that it could be used as an anti anaemic.

Acknowledgement

The authors gratefully acknowledge the technical assistance of Bitrus Wampana of Veterinary Physiology and Biochemistry, Isa of Veterinary Microbiology all of Faculty of Veterinary Medicine, University of Maiduguri and Fine Akawo of Chemistry Department.

Conflict of interest

None

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