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Original Article

Effect of *Acalypha Wilkesiana* Muell Arg. On Haematological Parameters In Wistar Albino Rats.

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ABSTRACT

The genus Acalypha comprises of about 570 species [1], *Acalypha wilkesiana* Muell Arg. belongs to the family Euphorbiaceae (spurge family). Its other names include *A. amentacea* and A. tricolor, while its common names are copperleaf, Joseph's coat, fire dragon, match-me-if-you-can. A large proportion of which are weeds while the other are ornamental plants. They are found all over the world most especially in the tropics of Africa, America and Asia. It is native to Fiji and nearby islands in the South Pacific. The weeds are wild and can be found everywhere while, the ornamental species must have been introduced into West Africa from other parts of the world and are cultivated as foliage plants in garden, greenhouse and parks. It is widely spread in the southern part of Nigeria. In most parts of Rivers State of Nigeria, traditional medicine has been claimed to be vital in preventing and curing various diseases, thereby playing an important role in the health services of the state especially among the low socio-economic class. In the coastal areas of Nigeria, the plant is used in the treatment of bacterial and fungal skin infections, and various gastrointestinal disorders [2-4], and also, used in the management of hypertension [5]. In view of its many uses, especially in Nigeria and the fact that traditional medicine practitioners prescribe and administer decoctions of the leaves to patients without regard to its possible adverse effects. The present investigation was undertaken to assess the effect of this plant leaves on haematological parameters in wistar albino rats. This is of important because there is no such information available in literature and scanty if available

1. Introduction

The genus Acalypha comprises of about 570 species [1], *Acalypha wilkesiana* Muell Arg. belongs to the family Euphorbiaceae (spurge family). Its other names include A. amentacea and *A. tricolor*, while its common names are copperleaf, Joseph's coat, fire dragon, match-me-if-you-can. A large proportion of which are weeds while the other are ornamental plants. They are found all over the world most especially in the tropics of Africa, America and Asia. It is native to Fiji and nearby islands in the South Pacific. The weeds are wild and can be found everywhere while, the ornamental species must have been introduced into West Africa from other parts of the world and are cultivated as foliage plants in garden, greenhouse and parks. It is widely spread in the southern part of Nigeria. In most parts of Rivers State of Nigeria, traditional medicine has been claimed to be vital in preventing and curing various diseases, thereby playing an important role in the health services of the state especially among the low socio-economic class. In the coastal areas of Nigeria, the plant is used in the treatment of bacterial and fungal skin infections, and various gastrointestinal disorders [2-4], and also, used in the management of hypertension [5]. In view of its many uses, especially in Nigeria and the fact that traditional medicine practitioners prescribe and administer decoctions of the leaves to patients without regard to its possible adverse effects. The present investigation was undertaken to assess the effect of this plant leaves on haematological parameters in wistar albino rats. This is of important because there is no such information available in literature and scanty if available

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2.Materials and Methods

2.1.Preparation of Plants Extract

Fresh samples of *A. wilkesiana* leaves were collected at 9:00 a.m. (GMT) from the herbal garden of Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The plant was identified and authenticated by a Taxonomist, Prof S.K. Adesina of Department of Pharmacognosy. The leaves were detached from the stems, washed twice with distilled water to remove adulterants, dried under natural conditions for two weeks, ground into powder using an electric blender and stored in airtight containers.

2.2.Animals

Thirty (30) male wistar albino rats weighing (180–190) g were obtained from the animal house unit of the Department of Pharmacology, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The animals were redistributed into stainless metabolic cages (five rats per cage) and observed under a 12- hour/12-hour light/dark cycle in a well-ventilated room at 26–27°C. They were fed with standard rat chow (Bendel Feed and Flour Mill Limited, Ewu, Benin City, Nigeria) and water *ad libitum*. The experimental protocol was in accordance with internationally accepted guidelines for animal use and care (EEC Directive of 1986; 86/09/EEC; National Institutes of Health publication 85-23, revised 1985). The acclimatization period lasted for 7 days.

2.3.Phytochemical screening

The phytochemical analysis of aqueous extract of Acalypha wilkensiana was found to contain tannins, triterpeniods, flavonoids, gallic acid, corilagin and geranin has been reported [6,7].

2.4.Experimental Design

Thirty (30) rats already obtained were acclimatized for a period of seven days, weighed, and divided into six groups comprising of five animals in each group. Animals were fed with substances under investigation for a period of twenty eight (28) days after which they were injected with 0.5 ml/kg CCL4, dissolved in 0.5 ml olive oil. They were fed ad libtium with free access to water. The groups are as follows:

Group1 : rats were fed with 100% rat feed and served as negative control
Group2 : rats were pretreated with 10% *Acalypha wilkensiana* +90% feed
Group3 : rats were pretreated with 30% *Acalypha wilkensiana* + 70% feed
Group4 : rats were pretreated with 50% *Acalypha wilkensiana* + 50% feed
Group5 : rats were pretreated with 30% *Acalypha wilkensiana* + 70% feed (positive control)
Group6 : rats were fed with rat feed and served as general control

Rats in groups (1-4), were injected with CCL_4 (0.5 ml/kg body weight in 0.5 ml olive oil), after feeding for twenty eight (28) days, and fasted for 24hrs.While rats in groups (5 and 6) were not administered with CCL_4 and served as positive and general controls respectively. The feeding method adopted in this experiment was based on previous work done by [8].

2.5.Sample collection

Twenty-four hours after the last administration, the animals were anaesthetized with chloroform vapour and dissected. Whole blood was obtained by cardiac puncture from each rat and collected into anticoagalant – treated (EDTA 0. 77M) sterile bottles. This was used for haematological studies. Blood haemoglobin (Hb) was determined spectrophotometrically by the cyanomethaemoglobin method,[9] packed cell volume (PCV) was determined by method of Jain, (1986).[9] Red Blood Cell Count (RBC), was estimated by haemocytometer method of (Jain, 1986; Dacie and Lewis, 2001).[9,10] Blood was diluted in 1,200 Dacie's fluid which keeps and preserves the integrity of the RBC. White Blood Cell (WBC) counts (Baker et al., 1985),[11] the dilution factor was 1:20 using 2-3% solution of acetic acid to which gentian violet was added. The calculations for red cell indices were made as described[9]

Mean Corpuscular Volume
MCV (fl) = PCV (1/1) / RBC count (×10-12)

Mean Cell Haemoglobin
MCH (pg) = Hb (g/dl) / RBC count (×/10-12/1)

Mean Cell Haemoglobin Concentration
MCHC (g/dl) = Hb (g/dl)/ PCV(1/1)

2.6.Statistical Analysis

The results were statistically analysed using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. P values<0.05 were considered significant.

3.Results

The effects of Acalypha wilkensiana on some haematological parameters: WBC, lymphocytes, neutrophils, eosinophils, monocytes, basophils and PCV, Hb, RBC, MCH, MCV, MCHC in CCl_4-induced hepatic injury in wistar albino rats are shown in Tables 1and 2, respectively. The results showed significant increase ($p \leq 0.05$) in the levels of WBC and neutrophils in rats received CCl_4 only. WBC and neutrophils levels were significantly reduced ($p \leq 0.05$) in rats that were pre-treated with 10% and 30% Acalypha wilkensiana (groups 2 and 3 respectively). However, the levels were still significantly raised when compared with the rats groups that were not administered with CCl_4 i.e. groups 5 and 6 (positive control and general control, respectively). Rats pre-treated with 50% Acalypha wilkensiana (group 4) showed significant increase ($p \leq 0.05$) in the levels of WBC and neutrophils. Administration of CCl_4 significantly decreased ($p \leq 0.05$) the levels of lymphocytes in

rats' group1, whereas there were significant increase ($p \leq 0.05$) in the levels of lymphocytes in rats that were pre-treated with 10% and 30% Acalypha wilkensiana (groups 2 and 3 respectively). However, there was a significant decrease in the levels of lymphocytes in rat groups that were pre-treated with 50% Acalypha wilkensiana. The eosinophils and monocytes levels showed no defined pattern between the groups, whereas basophils were absent in all the groups.

TABLE 1: The effects of *A. wilkensiana* on some haematological parameters in CCl4 induced Hepatotoxicity

TREATMENTS	WBC×10^9/L	L	N	E	M	B
100% FEED + Ccl_4	$10.50^a \pm 1.00$	$40.40^a \pm 11.0$	$57.80^a \pm 10.59$	$1.00^a \pm 0.01$	1.00 ± 0.01	-
90% FEED + 10% *A. wilkensiana* + Ccl_4	$8.50^b \pm 2.00$	$55.33^b \pm 5.84$	$44.00^b \pm 7.53$	$0.50^b \pm 0.01$	0.50 ± 0.01	-
70% FEED + 30% *A.wilkensiana* + Ccl_4	$7.45^c \pm 1.00$	$55.75^c \pm 1.00$	$42.50^c \pm 1.00$	$1.00^c \pm 0.01$	0.75 ± 0.01	-
50% FEED + 50% *A.wilkensiana* + Ccl_4	$9.80^d \pm 1.67$	$50.66^d \pm 7.41$	$48.00^d \pm 8.64$	$1.75^d \pm 0.01$	0.25 ± 0.01	-
70% FEED + 30% *A. wilkensiana* -CCl_4	$7.17^c \pm 0.74$	$62.50_e \pm 1.00$	$37.50^e \pm 1.00$	-	-	-
(positive control) 100% FEED – CCl_4 (General Control)	$6.05_e \pm 1.00$	$64.00_f \pm 2.40$	$36.00_e \pm 1.10$	-	-	-

Values are means ± S.D for 5 replicate (n=5) Means with different superscripts are significantly different at the 0.05 levels.

The results in Table 2 showed that there was a significant decrease ($p \leq 0.05$) in the levels of Hb, PCV and RBC in rats administered with CCl4 only. Whereas rats that were pre-treated with 10% and 30% *Acalypha wilkensiana* (groups 2 and 3), showed significant increase ($p \leq 0.05$) in the levels of Hb, PCV and RBC, when compared with the rats that were administered CCl4 only. However, rats group 4 that were pre-treated with 50% *Acalypha wilkensiana* had a significant decrease ($p \leq 0.05$) in the levels of Hb, PCV and RBC. There was no significant difference ($p \leq 0.05$) in the MCH values calculated in all the animal groups. The MCV levels significantly reduced ($p \leq 0.05$) in rats group1, whereas significant increases ($p \leq 0.05$) were shown in groups (2 and 3). MCHC showed significant increases ($p \leq 0.05$) in rats groups (1 and 4), when compared with the control groups (5 and 6). While, non-significant increase was calculated in rats groups (2 and 3).

TABLE 2:The effects of *A. wilkensiana* on some haematological parameters in CCl_4 Hepatotoxicity

TREATMENTS	Hb (g/dl)	PCV (vol%)	RBC×10^6µl	MCH (ρg)	MCV (µm^3)	MCHC (%)
100%Feed +Ccl4	$14.55^a \pm 1.47$	$43.75^a \pm 4.49$	$8.80^a \pm 1.10$	16.53 ± 1.00	$49.72^a \pm 2.00$	$33.26^a \pm 2.00$
90%Feed+10% *A. wilkesiana* + Ccl_4	$15.20^b \pm 1.51$	$50.33^b \pm 3.85$	$9.60^b \pm 1.10$	15.83 ± 1.00	$54.43^b \pm 2.00$	$30.20^b \pm 2.00$
70%Feed+30% *A.wilkesiana* + Ccl_4	$16.77^c \pm 1.28$	$57.00^c \pm 8.57$	$9.80^c \pm 1.00$	17.11 ± 1.00	$58.16^c \pm 2.00$	$29.42^b \pm 2.00$
50%Feed+50% *A. wilkesiana* + Ccl_4	$14.90^a \pm 0.10$	$45.67^a \pm 4.49$	$8.85^a \pm 1.00$	16.84 ± 1.00	$51.60^a \pm 2.00$	$32.63^a \pm 2.00$
70%Feed+30% *A. wilkesiana* –CCl_4	$18.00^d \pm 1.28$	$67.00^d \pm 1.00$	$10.50^d \pm 1.10$	17.14 ± 1.00	$63.81^d \pm 2.00$	$26.87^b \pm 2.00$
(positive control) 100%Feed – CCl_4 (General Control)	$18.65^d \pm 2.86$	$65.00^d \pm 1.00$	$10.50^d \pm 1.10$	17.76 ± 1.00	$61.90^d \pm 2.00$	$28.69^b \pm 2.00$

Values are means ± S.D for 5 replicates (n=5) Means with different superscripts are significantly different at the 0.05 levels.

4.Discussion

Haematological studies on some vital haematological indices were carried out during the chronic toxicity study in rats due to their roles in providing reliable information concerning haematological changes toxicants could cause. Haematological parameters have been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health [12]. The increased Hb, PCV and RBC values in the positive control group5 (30% Acalypha wilkensiana without CCl4) may be attributed to the beneficial effect of the herb at this dose while, general control (group6), in Tables 2, could be as a result of absence of toxicants (CCl4-free) in the diets, which also implies that there were little or no damage to the internal organs. The herb, Acalypha wilkesiana produced significant increases in PCV, Hb, RBC, MCV, lymphocytes and significant decreases in the levels of WBC and neutrophils in rats groups (2 and3) pretreated with 10% and 30% A. wilkesiana, respectively, when compared with rats administered with CCl4 only (group1). This agrees with the earlier report by [13] that xenobiotics can cause haemolytic anaemia when sulphydryl groups of the erythrocyte membrane is oxidized

which inflicts injury to the erythrocyte membrane. Nwinuka et al.[14] later reported the positive effect of the extract of Mangifera indica (stem bark) on the haemopoietic system of test rats. The haemorrage caused by CCl4 in liver was minimized by the pretreatment of Acalypha wilkensiana, as flavonoids, one of its active components are known to be vasculo- protector and powerful antioxidant [15]. The increased values of RBC and associated parameters Hb, PCV are suggestive of polycythemia. Therefore, A. wilkesiana at (10% and 30%) may not have had any adverse effect on the bone marrow, liver and haemological metabolism, since the values of red blood cells are not greatly affected [16]. The increases in WBC and neutrophils in CCl4 treated rats observed in this study (group1), may be considered as a defensive mechanism by the immune system which corroborate the report by[17] that when an antigen is introduced into an organism, antibodies are produced in response to the antigen. Neutrophils are the most abundant circulating granulocytes, their granules contain numerous microbicidal molecules, and when a chemo-tactic factor is produced because of infection or injury in an extracellular site, these cells enter the tissues (Weir and Stewart, 1999)[18]. The results of this study showed that rats pretreated with 50% Acalypha wilkesiana, appear to suppress the haemopoietic system. There was reduction in the haematological parameters Hb, PCV, RBC and lymphocytes when compared with the controls. The reduction may have occurred due to lysis of blood cells because of toxicity of the herb, which may be above the safe dose for rats. These findings may be in agreement with the earlier report by [19] who observed an adverse reaction to the ointment of Acalypha wilkesiana in the treatment of some superficial fungi diseases.

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