Effects Of *A. Congensis* Extract On Heamatology Of Albino Rat

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ABSTRACT

Purpose: The study was conducted to investigate the possible effects of *Alstonia congensis* (leaf, bark and root) extracts on heamatology of albino rat.

Is the extract poisonous? What is the LD₅₀ of the extract? Is the heamatology of the test animals affected by the extract? Is the effect dangerous for the animal?

Method: The study was carried out between February 2019 to October 2020. *A. congensis* was collected from Emekuku area of Imo State. The albino rats were purchased from the Animal Science and Production Department of Michael Okpara University of Agriculture, Umudike, Umuahia. Acute toxicity studies of the crude extracts were carried out in albino mice. The possible effects of the extracts on hematology of the albino rats were determined using automated machine.

Results: In acute toxicity test, all the mice that received the doses (10, 100, 1000, 1600, 2900 and 5000 mg/kg) of the extract survived beyond the 2 weeks of observation. The medium lethal dose toxicity value (LD₅₀) of the extract must be above 5000 mg/kg which indicates that the extract is safe for consumption. The extracts did exhibit some hematological changes, the packed cell volume (PCV) of the test animals were reduced. The RBC of the animals were reduced significantly when 800 mg/kg of bark extract was given, but no mortality was observed. The WBC and HB of the animals were affected but the effects were not significant. There was no evidence of drug-induced symptoms at all the doses of the extract administered.

Conclusion: The extract can be deleterious to the heamatological parameters of the test animal when used in high doses for a long period. Therefore, it is advisable to be used in chronic cases where orthodox medicine failed.

Keywords: Alstonia, dose, extract, haematology, toxicity.

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I. INTRODUCTION

Phytotherapeutic agents or phytomedicines are standardized herbal preparations consisting of complex mixture of one or more plants which are used in most countries for the management of various diseases. Usually, the active principles responsible for their pharmacological action are unknown. One basic characteristic of phototherapeutic agents is the fact that they normally do not possess an immediate or strong pharmacological action [1]. For this reason, phototherapeutic agents are not used for emergency treatment. Other characteristics of herbal medicine are their wide therapeutic use and great acceptance by the population. In contrast to modern medicines herbal medicines are frequently used to treat chronic diseases. Sub-acute toxicity evaluations are required to establish potential adverse effects of the medicinal preparations on internal organs [2]. A complete blood count (CBC), also known as a complete blood cell count, full blood count (FBC), or full

blood exam (FBE), is a blood panel requested that gives information about the cell in the blood, such as the cell count for each cell type and the concentrations of various proteins and minerals. Blood counts of various types have been used for clinical purposes since the 19th century. Automated equipment to carry out complete blood counts was developed in the 1950s and 1960s [3].

The cells that circulate in the bloodstream are generally divided into three types: white blood cells leukocytes red blood cells (erythrocytes) and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease. According to work carried out by [4], on Malaria anemia of mice and man, hematological abnormalities are considered a hallmark of malaria. Reference [5] reported that *P. berghei* increases erythrocyte fragility and significantly reduces PCV in rat and also affects the WBC which is an important index of pathological and physiological status [6].

II. MATERIALS AND METHODS

A. Extraction of Plant Materials

Fifty grams (50 g) of the pounded dried plant materials (leaf, bark and root powder) were weighed and extracted with 400 ml of aqueous (distilled water) and 400 ml of ethanol using [7], extraction method. The processes were run for 2 hours each after which the samples were evaporated to dryness using water bath. The dried extracts were weighed and kept in a well labeled sterile specimen bottles and stored in are refrigerator at 4 °C until is required.

B. Acute Toxicity/Lethal Dose (LD₅₀) Test

The medium lethal dose of the crude extracts of leaf, bark and root of *A. congensis* were determined by [8] method using the oral routes with the assistance of Pharmacist Solomon Nwafuru of Federal Medical Centre, Owerri. The acute oral toxicity study was conducted in compliance with OECD guideline 425, which stipulate the use of only three animals [9]. The test was divided into two stages.

Stage One: Determination of the toxic range of the leaf, bark and root extracts of *Alstonia congensis*. Albino rats were divided into 9 groups of 3 animals in each group. Each group received a dose (10, 100, 1000 mg/kg) of the ethanolic extracts of leaf, bark and root suspended in distilled water respectively. The doses were administered orally, and the treated animals observed for 72 hours for number of deaths.

Stage Two: Determination of lethality of leaf, bark and root extracts. The doses used in this stage were determined from the number of deaths per dose recorded in the stage one test. Since no death occurred in the stage one test, three different higher doses: 1600 mg/kg, 2900 mg/kg and 5000 mg/kg were administered to another group of animals at one dose per animal. The treated animals were monitored for number of deaths for 24 hours and continued to 72 hours. The LD₅₀ in this test is determined by calculating the geometric mean of the test and most toxic doses.

$$LD_{50} = \sqrt{\text{minimum toxic dose} \times \text{maximum tolerated dose}}$$

C. Sub-Acute Toxicity Studies

For the sub-acute toxicity studies, hematological parameters were determined in relation to the control treatment. These include packed cell volume (PCV), white blood cells (WBC), red blood count (RBC), haemoglobin (HB), platelets, etc. These investigations were carried out to determine the effect of the extract on internal organs using albino rat as the test animals. A low (400 mg/kg) and high 800 mg/kg of the extracts were used in this study.

D. Data Analysis

Data were analyzed using computer software SPSS, Version 16. Results of the study were expressed as a mean $\pm$ standard error of the mean ($m \pm SEM$). Statistical significance was determined by one way analysis of variance (ANOVA) with multiple comparison tests (Post Hoc/Tukeys test/HSD) to compare parameters within groups. All data were analyzed at a 95% confidence interval ($\alpha = 0.05$).

III. RESULTS

A. Pre-Treatment Acute Toxicity/Lethality Test

The result of the lethality and acute toxicity studies of the leaf, bark and root extract of *Alstonia congensis* in a naïve rat are shown below.

TABLE I: ACUTE TOXICITY (LD₅₀) TEST OF THE CRUDE LEAF EXTRACT OF *A. congensis*

Stages	Doses mg/kg	Mortality
Stages 1	10	0/3
	100	0/3
	1000	0/3
Stages 2	1600	0/1
	2900	0/1
	5000	0/1

TABLE II: ACUTE TOXICITY (LD₅₀) TEST OF THE CRUDE LEAF EXTRACT OF *A. congensis*

Stages	Doses mg/kg	Mortality
Stages 1	10	0/3
	100	0/3
	1000	0/3
Stages 2	1600	0/1
	2900	0/1
	5000	0/1

TABLE III: ACUTE TOXICITY (LD₅₀) TEST OF THE CRUDE LEAF EXTRACT OF *A. congensis*

Stages	Doses mg/kg	Mortality
Stages 1	10	0/3
	100	0/3
	1000	0/3
Stages 2	1600	0/1
	2900	0/1
	5000	0/1

TABLE IV: SHOWING THE EFFECT OF THE EXTRACTS OF *A. congensis* PARTS ON THE HAEMATOLOGICAL WBC AND HB OF TEST ANIMALS

Parameters	Treatment	Mean values	Significance
WBC (x 10 ³ /μL)	Normal (control)	19.42 $\pm$ 2.83	-
	400 mg/kg Leaf	24.42 $\pm$ 4.87	P > 0.05
	800 mg/kg Leaf	19.13 $\pm$ 4.18	P > 0.05
	400 mg/kg Bark	19.83 $\pm$ 2.58	P > 0.05
	800 mg/kg Bark	16.85 $\pm$ 2.10	P > 0.05
	400 mg/kg Root	15.98 $\pm$ 1.69	P > 0.05
	800 mg/kg Root	19.00 $\pm$ 2.44	P > 0.05
	20 mg/kg Artesunate	17.77 $\pm$ 1.76	-
Hb (g/dl)	Normal (control)	14.27 $\pm$ 0.42	-
	400 mg/kg Leaf	14.12 $\pm$ 0.51	P > 0.05
	800 mg/kg Leaf	13.73 $\pm$ 0.45	P > 0.05
	400 mg/kg Bark	14.25 $\pm$ 0.68	P > 0.05
	800 mg/kg Bark	12.57 $\pm$ 0.67	P < 0.05
	400 mg/kg Root	13.90 $\pm$ 0.34	P > 0.05
	800 mg/kg Root	14.07 $\pm$ 0.63	P > 0.05
	20 mg/kg Artesunate	14.00 $\pm$ 0.13	-

The results are expressed as mean $\pm$ SEM, P>0.05 is not significant, n = 10.

At respective doses of 10, 100 and 1000 mg/kg, as shown in tables (i, ii & iii), all the three animals given the extracts of leaf, bark and root survived beyond the two weeks of observation without any sign of illness. When the extract was increased to 1600, 2900 and 5000 all the animals equally survived. All the rats that received the doses (10, 100, 1000, 1600, 2900 and 5000 mg/kg) of the extract survived beyond the 2 weeks of observation. The medium lethal dose toxicity value (LD₅₀) of the extract must be above 5000 mg/kg. There were no gross physical and behavioral changes including

rigidity, sleep, diarrhea, depression, abnormal secretion, and hair erection within the observation period.

The mean WBC and HB (table iv) of the animal were 19.42 ± 2.83 and 14.27 ± 0.42 before the extract were given. When 400 mg/kg and 800 mg/kg doses of leaf extract were given the mean WBC and HB of the animal were 24.42 ± 4.87 , 14.12 ± 0.51 and 19.13 ± 4.18 , 13.73 ± 0.45 respectively. When 400 mg/kg of bark was given, WBC and Hb were 19.83 ± 2.58 , 14.25 ± 0.68 and 16.85 ± 2.10 , 12.57 ± 0.674 respectively. For 400 mg and 800 mg/kg of root; the WBC and Hb were 15.98 ± 1.69 , 13.90 ± 2.34 and 19.00 ± 2.44 , 14.07 ± 0.63 .

The WBC of the animal was high when 400 mg/kg of leaf was given and low when 400 mg/kg of root was given but the change in the WBC of all the test animal was not significant when compared to the normal.

The Hb of the animals for all the extract doses dropped below normal but not statistically significant except for 800 mg/kg of bark which was significant.

TABLE V: SHOWING THE EFFECT OF EXTRACTS OF *A. congensis* PARTS ON RBC AND PCV OF ALBINO RAT

Parameters	Treatment	Mean values	Significance
RBC (x 103/ μ L)	Normal (control)	7.44 ± 0.29	-
	400 mg/kg Leaf	8.27 ± 0.60	$P > 0.05$
	800 mg/kg Leaf	7.47 ± 0.24	$P > 0.05$
	400 mg/kg Bark	7.77 ± 0.28	$P < 0.05$
	800 mg/kg Bark	6.72 ± 0.37	$P > 0.05$
	400 mg/kg Root	7.35 ± 0.15	$P > 0.05$
	800 mg/kg Root	7.70 ± 0.39	$P > 0.05$
	20 mg/kg Artesunate	8.40 ± 0.37	-
	Normal (control)	47.95 ± 0.83	-
	400 mg/kg Leaf	45.63 ± 1.02	$P < 0.05$
PCV (%)	800 mg/kg Leaf	41.92 ± 1.43	$P < 0.05$
	400 mg/kg Bark	46.08 ± 1.43	$P < 0.05$
	800 mg/kg Bark	40.50 ± 1.05	$P < 0.05$
	400 mg/kg Root	42.22 ± 1.17	$P < 0.05$
	800 mg/kg Root	44.02 ± 1.42	$P < 0.05$
	20 mg/kg Artesunate	42.97 ± 0.85	-

The results are expressed as mean $\pm$ SEM $P < 0.05$ is significant, $n = 10$.

TABLE VI: RESULTS SHOWING THE EFFECT OF EXTRACTS OF *A. congensis* PARTS ON MCV AND MCH OF TEST ANIMALS

Parameters	Treatment	Mean values	Significance
MCV (f/l)	Normal (control)	64.55 ± 2.15	-
	400 mg/kg Leaf	55.38 ± 3.21	$P < 0.05$
	800 mg/kg Leaf	56.17 ± 3.09	$P < 0.05$
	400 mg/kg Bark	59.40 ± 2.59	$P < 0.05$
	800 mg/kg Bark	60.50 ± 4.52	$P < 0.05$
	400 mg/kg Root	57.45 ± 1.78	$P < 0.05$
	800 mg/kg Root	57.28 ± 3.41	$P < 0.05$
	20 mg/kg Artesunate	51.21 ± 1.88	-
	Normal (control)	19.21 ± 0.84	-
	400 mg/kg Leaf	17.19 ± 1.92	$P < 0.05$
MCH (p/g)	800 mg/kg Leaf	18.39 ± 0.72	$P > 0.05$
	400 mg/kg Bark	18.38 ± 1.17	$P > 0.05$
	800 mg/kg Bark	18.78 ± 1.98	$P > 0.05$
	400 mg/kg Root	18.92 ± 0.51	$P > 0.05$
	800 mg/kg Root	18.29 ± 0.79	$P > 0.05$
	20 mg/kg Artesunate	16.70 ± 0.80	-

The results are expressed as mean $\pm$ SEM $P > 0.05$ is not significant, $n = 10$, $P < 0.05$ is significant

The RBC and PVC of the test animals (table v) before the treatment were 7.44 ± 0.29 and 47.95 ± 0.83 , when 400 and 800 mg/kg of the leaf extract were given, the RBC and PVC of the animals were 8.27 ± 0.60 , 45.63 ± 1.02 and 7.47 ± 0.24 ,

41.92 ± 1.43 respectively. For 400 and 800 mg/kg of bark extracts, the RBC and PCV were 7.77 ± 0.37 , 46.08 ± 1.43 and 6.72 ± 0.37 , 40.50 ± 1.05 respectively. When 400 and 800 mg/kg of root extracts were given, RBC and PCV were 7.35 ± 0.15 , 42.02 ± 1.17 and 7.70 ± 0.39 , 44.02 ± 1.42 respectively. The RBC of the animals were reduced significantly when 800 mg/kg of bark extracts was given. The PCV of all the animals were significantly reduced at all the doses of the extracts.

The results of the effect of extracts of leaf, bark and root of *A. congensis* on MCV and MCH of test animals were shown in Table VI. When 400 mg/kg and 800 mg/kg of leaf extract were given, the values for MCV and MCH were 55.38 ± 3.21 , 17.19 ± 1.92 , and 56.17 ± 3.09 , 18.39 ± 0.72 respectively. For 400 and 800 mg/kg of bark, the values of MCV and MCH were 59.40 ± 2.59 , 18.38 ± 1.17 and 60.50 ± 4.52 , 18.78 ± 1.98 , respectively.

The values at 400 and 800 mg/kg of root were 57.45 ± 1.78 , 18.92 ± 0.51 and 57.28 ± 3.41 , 18.29 ± 0.79 respectively. All the extracts significantly reduced the MCV of the tested animals but the MCH was significantly reduced when 400 mg/kg of leaf extract was given.

TABLE VII: RESULTS SHOWING THE EFFECT OF EXTRACTS OF *A. congensis* PARTS ON MCHC AND PLATELET OF TEST ANIMALS

Parameters	Treatment	Mean values	Significance
MCHC (g/dl)	Normal (control)	29.76 ± 0.92	-
	400 mg/kg Leaf	30.96 ± 1.73	$P > 0.05$
	800 mg/kg Leaf	32.78 ± 0.96	$P < 0.05$
	400 mg/kg Bark	30.93 ± 1.34	$P > 0.05$
	800 mg/kg Bark	31.02 ± 1.64	$P > 0.05$
	400 mg/kg Root	32.96 ± 1.51	$P < 0.05$
	800 mg/kg Root	32.01 ± 2.30	$P < 0.05$
	20 mg/kg Artesunate	32.60 ± 0.89	-
	Normal (control)	728.33 ± 8.31	-
	400 mg/kg Leaf	638.17 ± 81.88	$P < 0.05$
PLATELET	800 mg/kg Leaf	419.00 ± 12.03	$P < 0.05$
	400 mg/kg Bark	635.50 ± 23.31	$P < 0.05$
	800 mg/kg Bark	540.67 ± 12.68	$P < 0.05$
	400 mg/kg Root	532.17 ± 45.94	$P < 0.05$
	800 mg/kg Root	615.33 ± 4.13	$P < 0.05$
	20 mg/kg Artesunate	684.50 ± 14.24	-

The results are expressed as mean $\pm$ SEM $P < 0.05$ is significant, $n = 10$.

The results of the effect of extracts of leaf, bark and root of *A. congensis* on MCHC and platelet of test animals were shown in Table VII. The MCHC and platelet of the test animals before the treatment were 29.76 ± 0.92 and 728.32 ± 8.31 , respectively.

When 400 mg/kg and 800 mg/kg of leaf extracts were given, the values for MCHC and platelet were 30.96 ± 1.73 , 638.17 ± 81.88 and 32.78 ± 0.96 , 419.00 ± 12.03 , respectively.

For 400 mg/kg and 800 mg/kg of bark, the values of MCHC and platelets were 30.93 ± 1.34 , 635.50 ± 23.31 and 31.02 ± 1.64 and 540.67 ± 12.68 , respectively.

The values at 400 and 800 mg/kg of root were 32.96 ± 1.51 , 532.17 ± 45.93 and 32.01 ± 2.30 , 615.33 ± 4.13 respectively. The extracts in all the doses significantly reduced the platelet of the test animals but the MCHC was significantly increased when 800 mg/kg of leaf, 400 and 800 mg/kg of root extracts

were given.

IV. DISCUSSION

Plants in general constitute a wide array of phytochemicals and when used in high dosages can elicit harmful effect on the body [10]. According to [11], *A. congensis* has frequently been used as antiparasmodial with great positive result but have been relatively uninvestigated for possible adverse effect on hematology. Based on this, the study was conducted to investigate the adverse effect of *A. congensis* preparations on hematological parameters of albino rat. In the acute toxicity study of the extract, no changes in the behavior was observed. All the mice that received the doses of the extracts survived beyond the 2 weeks of observation. This was in agreement with the study carried out by [12], in which the extract of *A. congensis* and *X. aethiopica* fruits showed no behavioral changes in mice treated with 20 mg/kg dose of the extract.

In this present study, *A. congensis* preparations reduced the PCV and RBC of the rat which also agrees with the work of [5]. The hematological parameters of treated animals (Hb, PCV) are index of anaemia. There was statistically significant difference in PCV of the treated animals from the control for all the doses of the extracts. The reduction in PCV and RBC may result to anaemia when used for long term.

Similarly, WBC which is an important index of pathological and physiological status [6], exhibited no significant difference, which implies that the presence of the phytochemicals which is for defense in plants, work in line with the WBC of the animals which is also for defense.

It would therefore be worthwhile to standardize the extract to know the dose and the time frame in order not to cause any adverse effect on the internal organs of the animals.

V. CONCLUSION

The high dose of the extracts should not be used for long time. The dosage range should not exceed 4 to 7 days to avoid any deleterious effect on the internal organ as well as cause anemia because of its effect on hematological parameters. The extract may be preferable for chronic infections where orthodox medicine failed.

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