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 LD$_{50}$ 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 heamatology 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 (LD$_{50}$) 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 hematological 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, heamatology, toxicity.

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I. INTRODUCTION

Phytherapeutic 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. Subacute 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 Alstonia 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.

LD₅₀=√minimum toxic dose×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 ± standard error of the mean (m ± 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             |
| Stages 1        | 100             | 0/3             |
| Stages 1        | 1000            | 0/3             |
| Stages 2        | 1600            | 0/1             |
| Stages 2        | 2900            | 0/1             |
| Stages 2        | 5000            | 0/1             |

| TABLE II: ACUTE TOXICITY (LD₅₀) TEST OF THE CRUDE BARK EXTRACT OF A. congensis |
|-----------------|-----------------|-----------------|
| Stages          | Doses mg/kg     | Mortality       |
| Stages 1        | 10              | 0/3             |
| Stages 1        | 100             | 0/3             |
| Stages 1        | 1000            | 0/3             |
| Stages 2        | 1600            | 0/1             |
| Stages 2        | 2900            | 0/1             |
| Stages 2        | 5000            | 0/1             |

| TABLE III: ACUTE TOXICITY (LD₅₀) TEST OF THE CRUDE ROOT EXTRACT OF A. congensis |
|-----------------|-----------------|-----------------|
| Stages          | Doses mg/kg     | Mortality       |
| Stages 1        | 10              | 0/3             |
| Stages 1        | 100             | 0/3             |
| Stages 1        | 1000            | 0/3             |
| Stages 2        | 1600            | 0/1             |
| Stages 2        | 2900            | 0/1             |
| Stages 2        | 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 10³/µL)  | Normal (control)| 19.42 ± 2.83    | -            |
| 400 mg/kg Leaf  | 4.24 ± 4.87     | P > 0.05        |
| 800 mg/kg Leaf  | 19.13 ± 4.18    | P > 0.05        |
| 400 mg/kg Bark  | 19.83 ± 2.58    | P > 0.05        |
| 800 mg/kg Bark  | 16.85 ± 2.10    | P > 0.05        |
| 400 mg/kg Root  | 15.98 ± 1.69    | P > 0.05        |
| 800 mg/kg Root  | 19.00 ± 2.44    | P > 0.05        |
| 20 mg/kg Artesunate | 17.77 ± 1.76  | -              |
| Hb (g/dl)       | Normal (control)| 14.27 ± 0.42    | -            |
| 400 mg/kg Leaf  | 14.12 ± 0.51    | P > 0.05        |
| 800 mg/kg Leaf  | 13.73 ± 0.45    | P > 0.05        |
| 400 mg/kg Bark  | 14.25 ± 0.68    | P > 0.05        |
| 800 mg/kg Bark  | 12.57 ± 0.67    | P < 0.05        |
| 400 mg/kg Root  | 13.90 ± 0.34    | P > 0.05        |
| 800 mg/kg Root  | 14.07 ± 0.63    | P > 0.05        |
| 20 mg/kg Artesunate | 14.00 ± 0.13  | -              |

The results are expressed as mean ± 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 was 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 19.83±2.58, 14.12±0.51 and 19.13±4.18, 13.73±0.45 respectively. When 400 mg/kg 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.

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.

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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 antimalarial 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 anemia. 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 anemia 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.

REFERENCES

[1] Akerele O, Heywood V, Syngê H. The conservation of medicinal plants. Cambridge University Press, Cambridge, UK. 1991.
[2] Rhiouani H, El-Hilaly J, Israël ZH, Lyoussi B. Acute and sub-chronic toxicity of an aqueous extract of the leaves of Hermiaria glabra in rodents. Journal of Ethnopharmacology. 2008; 118: 378-386.
[3] Verso ML. The Evolution of Blood Counting Techniques Read at a meeting of the Section of the History of Medicine, First Australian Medical Congress. 1962; 8: 149–58.
[4] Lamkanna AA, Brown D, Potocnik A, Casals-Pascual C, Langhorne J, Roberts DJ. Malaria anemia of mice and men. Blood. 2007; 110: 18-28.
[5] Iyawe HOT, Onigbinde AO. Impact of Plasmodium berghei and chloroquine on Haema antioxidant indices in mice. Asian Journal of Biotechnology. 2009; 4: 30-33.
[6] Mengistie B, Makonnen E, Urga K. In vivo antimalarial activity of Dodonaea angustifolia seed extracts against Plasmodium berghei in mice model. MEJS. 2012; 4: 47-63.
[7] Tédong L, Dzeufiet PDD, Dimo T, Asongalem EA, Sokeng SN, Flejou JF, et al. Acute and Subchronic toxicity of Anacardium occidentale Linn (Anacardiaceae) leaves hexane extract in mice. African Journal of Traditional Alternative Medicine. 2007; 4(2): 140-147.
[8] Lorkes D. A new approach for acute toxicity testing. Arch Toxicology. 1983; 54: 275-289.
[9] Jonsson M, Jestoi M, Nathanael AV, Kokkonen UM, Anttila M, Koivisto P, et al. Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. Food Chem Toxicology. 2013; 53: 2732.
[10] Wang MW, Hao X, Chen K. Biological screening of natural products and drug innovation in China. Philosophical Transactions of the Royal Society Biological Sciences. 2007; 362, 1093-1105.
[11] Dike JP, Obereme OO. Towards the conservation of Nigeria’s indigenous medicinal plants. Journal of Medicinal Plants Research. 2012; 6: 3517–3521.
[12] Ogbonnia S, Addenlade AA, Bosa MK, Enwuru VN. Evaluation of acute and sub acute toxicity of A. congensis bark and xylia aethiopica fruit mixtures used on the treatment of diabetes. African journal of Biotechnology. 2008; 7(6): 701-705.

DOI: http://dx.doi.org/10.24018/ejmed.2022.4.1.1083