

Hematological effects of repeated graded doses of the methanol extract of *Paullinia pinnata* (Linn.) leaves in Wistar albino rats

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ABSTRACT

Background: *Paullinia pinnata* is a medicinal plant used for the treatment of various diseases, including anemia in West Africa. **Aim:** This study was carried out to investigate the effect of increasing doses of the methanolic leaves extract of *P. pinnata* on hematological parameters in rats. **Materials and Methods:** Thirty-six male Wistar albino rats were grouped into six groups of six animals each. Five doses; 50, 100, 200, 400 and 800 mg/kg body weight of the *P. pinnata* extract were administered separately to five groups. The sixth group served as a control and received only the vehicle (70% physiological saline: 30% Tween 80 [v/v]). Administration was done orally daily for 28 days at 24 h interval. On day 29, the animals were made inactive, blood was then collected from the heart and various hematological parameters were evaluated. **Statistical Analysis:** Analysis of variance was employed. **Results:** The packed cell volume and red blood cell count increased significantly ($P < 0.05$) in the treatment groups except at 200 mg/kg dose. The hemoglobin concentration increased in all the treatment groups. The values for the neutrophils at 50, 100, 200 and 800 mg/kg doses were higher than that of the control. The white blood cell count increased significantly ($P < 0.05$) at 50 and 400 mg/kg doses compared to the control and exceeded the normal physiological range. **Conclusion:** The maximum tolerable dose is 200 mg/kg body weight of the methanolic leaves extract of *P. pinnata* and the extract has anti-anemic property with the ability to increase neutrophils count.

Key words: Erythropoiesis, hematological parameters, neutrophils, *Paullinia pinnata*

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INTRODUCTION

Paullinia pinnata is a woody or sub woody climber in damp sites in the Savannah zone. It belongs to the plant family Sapindaceae and grows throughout West Tropical Africa and Madagascar.^[1] It has no known common name, but its local names include Kakansela-Nigeria (Yoruba) and Toa-ntini-Ghana (Asante-Twi).^[1,2] Traditionally, the leaf is taken copiously with other herbs for the treatment of various ailments including anemia.^[1]

In this preliminary report, our aim is to validate the folkloric claim that the leaf extract of *P. pinnata* has erythropoietic potential.

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MATERIALS AND METHODS

Animal experiment and ethical review

A method for the preparation of the animals, group size and mode of administration was patterned after the experimental design of Karrow *et al.*^[3]

Reagents

The reagents used were products from Sigma-Aldrich, U.S.A. or Sure Chem, U.K and this include Drabkin's reagent, sodium citrate, acetic acid and Leishman's stain.

Plant sample

The leaves of *P. pinnata* were collected from the Forestry Research Institute of Nigeria, Ibadan, Nigeria. The plant was authenticated at the same Institute with the specimen voucher number FHI 106555.

Preparation and extraction of plant materials

The leaves were air-dried, milled, weighed and extracted in absolute methanol for 6 h in a soxhlet extractor over a

steam bath. The extract was pooled and concentrated to constant weight using a rotary evaporator (Heidolph HB, Germany) and a vacuum oven (Gallenkamp, England) at a temperature of 40–42°C.

Preparation of animals

Thirty-six healthy male Wistar albino rats whose body weight ranged from 140 to 190 g were used for the study. They were obtained from the central animal house, College of Medicine, University of Ibadan, Ibadan. They were kept in the Animal House, University of Ibadan, Nigeria to acclimatize. They were given feed (Guinea Feed Livestock Limited, Nigeria) and laboratory water *ad libitum* before starting the experiment. They were weighed and allocated into six groups of six animals each. The 12-h light/dark cycle was maintained.

Repeated graded dose administration study

Previous acute toxicity study showed that the extract was not toxic in male Wistar albino mice, even at 10,000 mg/kg body weight.^[4]

On day 1, five different doses of the methanol extract of the leaves of *P. pinnata* were administered orally to five of the groups separately. The administration was done daily to the rats at 24 h interval for 28 days. The doses were 50, 100, 200, 400 and 800 mg/kg body weight. The vehicle (physiological saline with Tween 80 [70:30 v/v]) was administered in the same manner to the sixth group and this served as a control. On day 29, the animals were made inactive by cervical dislocation under light ether anesthesia. The animals were then cut open and blood was collected from the heart using a needle and 5 ml syringe into ethylene diamine tetra acetic acid (EDTA) specimen bottles.

Determination of packed cell volume

The microhematocrit method of Baker and Silverton^[5] was employed. The procedure is as follows: A plain capillary tube was used to pick anti-coagulated blood from the EDTA specimen bottle. The lower end of the tube was sealed with plasticine and was then placed on a microhematocrit centrifuge (Hawksley, England). After centrifugation for 5 min at a speed of 30,000 rpm, the packed cell volume (PCV) was determined by measuring the height of the red blood cell (RBC) column with a PCV reader and this was expressed as a percentage of the height of the total blood column.

Determination of hemoglobin concentration

The cyanmet hemoglobin (Hb) method of Baker and Silverton^[5] was also employed. The procedure is as follows: 20 µl of blood was added to 4 ml of Drabkin's solution. The absorbance of this solution with the blood was read using a colorimeter (Gulfex, England) at a wavelength of

540 nm after mixing by inversion several times and allowed to stand at room temperature for 10 min. An ampoule of cyanmet Hb standard was opened, and the absorbance was read in the same colorimeter against the reagent blank. The final Hb concentration was then calculated.

Determination of red blood cell count

The procedure of Dacie and Lewis^[6] was used and is as follows: A 1 in 200 dilution of the blood sample was made in formol-citrate solution. The solution was made up by adding 10 ml of formalin (40% formaldehyde) to a 1 L solution of 32 g/L sodium citrate. 0.02 ml of each blood sample was added to 4.0 ml of diluent (formol-citrate solution), mixed thoroughly and then loaded into an improved Neubauer counting chamber. The RBC count for the whole blood sample was then calculated.

Determination of white blood cell count

The method of Dacie and Lewis^[6] was used and is as follows: 2% acetic acid-tinged with gentian violet was used as diluent. 0.02 ml of the blood sample was added to 0.38 ml of diluent to give a final dilution of 1 in 20. The diluted sample was then mixed, and a Pasteur pipette was used to load the sample on the counting chamber. The white blood cell (WBC) count for the whole blood sample was then calculated.

Determination of the mean cell hemoglobin concentration

This was calculated from the Hb concentration and PCV values (Baker and Silverton).^[5]

Determination of mean cell hemoglobin

This was calculated from the Hb concentration and the RBC count (Baker and Silverton).^[5]

Determination of the mean cell volume

This was calculated from the PCV and the RBC count (Baker and Silverton).^[5]

Differential leucocyte count

This was performed on thin blood films which were prepared on slides by the spread technique as reported by Baker and Silverton.^[5] The slides were then stained with Leishmann's stain and allowed to fix for 2 min and then diluted with 1 ml of buffered distilled water (pH 6.8) (49.6 ml of 0.067M Na₂HPO₄ [anhydrous] and 50.4 ml 0.067M KH₂PO₄ [anhydrous]) and allowed to stain for 10 min. The stain was then rinsed off with the buffered distilled water and then kept on the bench in a slant position. After drying, a drop of immersion oil was placed on the slide and the film examined with a ×100 objective on a binocular microscope (Olympus, Japan). The cells were counted using the longitudinal method by counting the different

types of white cells seen in one complete longitudinal strip of the film. If <100 cells are counted, a second strip was similarly enumerated. The result of each cell type was then expressed as a percentage. Nuclei of leucocytes stain purple; eosinophilic granules stain orange red; lymphocytes stain dark blue nuclei with pale blue cytoplasm; RBCs stain salmon pink; neutrophils stain lilac and cytoplasm of monocytes stain pale grey blue with Leishmann's stain.

Statistical analysis

One-way analysis of variance was employed in analyzing the results using the Predictive Analytics Software (International Business Machines (IBM), United States) Statistics 18 package. All the results were expressed as mean $\pm$ standard error and $P < 0.05$ was taken to be significant.

RESULTS

A 14% yield of the extract was realized from the initial weight of 1.2 kg of the air-dried leaf samples of *P. pinnata*.

The hematological parameters of the control and Wistar rats to which were administered varied doses of *P. pinnata* extract are shown in Table 1. The PCV increased significantly ($P < 0.05$) at 50, 100 and 400 mg/kg body weight doses while there was a significant decrease at the 200 mg/kg body weight dose compared to the control. The increase in PCV observed at the 800 mg/kg dose was not significant. The Hb concentration increased in all the treatment groups compared with the control with an increase at the 200 mg/kg dose not being significant ($P < 0.05$). The values for the RBC count increased significantly ($P < 0.05$) in all the treatment groups with the exception of the 200 mg/kg body weight dose, which showed a significant decrease compared to the control. With the exception of the 100 and 800 mg/kg body weight doses which showed a significant ($P < 0.05$) decrease in the WBC count, all the other doses showed a significant ($P < 0.05$) increase compared with the control and the highest increase was at 400 mg/kg body weight.

The hematological indices of treated and control rats are presented in Table 2. The mean cell Hb concentration (MCHC) decreased significantly ($P < 0.05$) in all the treatment groups compared to the control. The mean cell volume (MCV) increased in a dose-dependent manner from 100 to 400 mg/kg body weight doses and was significant ($P < 0.05$) in the groups treated with 200 and 400 mg/kg doses. The MCH increased at 100 and 200 mg/kg doses but was significant ($P < 0.05$) at the 200 mg/kg body weight dose, and decreased significantly at the 800 mg/kg dose compared to the control.

Table 3 revealed that in the groups treated with 50, 200 and 800 mg/kg doses, there were significant ($P < 0.05$) increases in the neutrophils compared to the control while the group treated with 400 mg/kg dose showed a significant decrease. The increase at the 100 mg/kg dose was not significant. The lymphocytes showed a significant ($P < 0.05$) increase at 100 and 400 mg/kg doses while the eosinophils decreased significantly ($P < 0.05$) in all the treatment groups compared to the control. The values for the monocytes decreased significantly in the groups treated with the 100, 200 and 400 mg/kg body weight doses compared to the control while it increased significantly in the group treated with the 800 mg/kg body weight dose.

Table 1: Hematological parameters of control and groups treated with graded doses of the methanolic leaves extract of *Paullinia pinnata*

Dose (mg/kg)	PCV (%)	Hb (g/100 ml)	RBC ($\times 10^{12}/L$)	WBC (/mm ³)
Control	52.67 $\pm$ 1.08	16.34 $\pm$ 0.33	5.43 $\pm$ 0.09	7.57 $\pm$ 1.34
50	55.17 $\pm$ 4.22*	17.50 $\pm$ 1.34*	5.83 $\pm$ 0.27*	9.53 $\pm$ 1.48*
100	60.67 $\pm$ 2.04*	18.36 $\pm$ 0.63*	5.64 $\pm$ 0.20*	6.84 $\pm$ 0.59*
200	50.92 $\pm$ 5.66*	16.39 $\pm$ 1.14	5.33 $\pm$ 0.31*	8.43 $\pm$ 1.33*
400	61.63 $\pm$ 2.66*	18.63 $\pm$ 0.81*	6.05 $\pm$ 0.24*	10.26 $\pm$ 1.37*
800	53.40 $\pm$ 2.10*	16.75 $\pm$ 0.63*	5.60 $\pm$ 0.81*	6.31 $\pm$ 0.27*

n=6. *Significant at $P < 0.05$. PCV=Packed cell volume; Hb=Hemoglobin concentration; RBC=Red blood cell count; WBC=White blood cell count

Table 2: Hematological indices of control and groups treated with graded doses of the methanolic leaves extract of *Paullinia pinnata*

Dose (mg/kg)	MCHC (g/100 ml)	MCV (fl)	MCH (pg)
Control	32.68 $\pm$ 0.05	96.57 $\pm$ 0.76	31.67 $\pm$ 0.65
50	31.33 $\pm$ 0.03*	96.57 $\pm$ 4.41	31.38 $\pm$ 1.49
100	31.35 $\pm$ 0.03*	97.10 $\pm$ 3.25	32.17 $\pm$ 1.83
200	31.31 $\pm$ 0.10*	99.50 $\pm$ 5.32*	33.38 $\pm$ 1.49*
400	31.33 $\pm$ 0.05*	100.29 $\pm$ 1.13*	31.49 $\pm$ 0.48
800	31.42 $\pm$ 0.07*	95.63 $\pm$ 1.11	28.67 $\pm$ 0.05*

n=6. *Significant at $P < 0.05$. MCHC=Mean cell hemoglobin concentration; MCV=Mean cell volume; MCH=Mean cell hemoglobin

Table 3: Differential leukocyte count in control and groups treated with graded doses of the methanolic leaves extract of *Paullinia pinnata*

Dose (mg/kg)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)
Control	16.25 $\pm$ 2.90	76.66 $\pm$ 2.98	3.28 $\pm$ 0.48	1.48 $\pm$ 0.29
50	19.56 $\pm$ 1.93*	75.46 $\pm$ 2.78	2.69 $\pm$ 0.48*	1.46 $\pm$ 0.50
100	16.76 $\pm$ 1.89	80.33 $\pm$ 1.47*	2.26 $\pm$ 0.25*	1.10 $\pm$ 0.41*
200	19.27 $\pm$ 2.99*	76.04 $\pm$ 3.59	2.52 $\pm$ 0.50*	0.76 $\pm$ 0.48*
400	15.22 $\pm$ 2.63*	81.54 $\pm$ 2.81*	2.00 $\pm$ 0.00*	1.32 $\pm$ 0.75*
800	19.83 $\pm$ 2.94*	75.75 $\pm$ 3.66	2.47 $\pm$ 0.50*	1.72 $\pm$ 0.25*

n=6. *Significant at $P < 0.05$

DISCUSSION

The findings of this preliminary investigation showed that the methanolic leaves extract of *P. pinnata* may have erythropoietic capacity, and it could also improve neutrophils count.

The results of the treated groups were compared with observed physiological ranges in normal animals (as reported by Mitruka and Rawnsley)^[7] and control. PCV, which is also known as the hematocrit, is a measure of the volume of the red cells (erythrocytes) in the blood. In the rats treated with 50, 100 and 400 mg/kg body weight doses, the PCV values showed that blood volume was adequate and even higher than that of the control. The values for the PCV observed in the control and the treatment groups were above the physiological range for normal rats (42.50–49.40%).^[7] Similarly, Hb concentration was also higher in the treated rats than in the control group which may be an indication of adequate blood formation and pigmentation.^[8] This implies that the oxygen carrying capacity of the blood was higher in the treated rats than in the control. This may be attributed to the extract having the ability to enhance erythropoiesis. Moreover, the groups treated with 50, 100 and 400 mg/kg doses had higher levels of Hb concentration than the upper limit of the physiological range for normal rats (12.00–17.50 g/100 ml).^[7] This is similar to the results of Obeagu *et al.*^[9] and Egba *et al.*^[10] using the leaf extract of *Telfairia occidentalis* and the observations of Kadham^[11] in rabbits using the aqueous extract of *Haloxylan salicornicum*. The treated rats recorded higher values for erythrocytes than the control with the exception of those treated with 200 mg/kg dose. This compares well with the results of Dharmarathna *et al.*^[12] which showed that the leaf extract of *Carica papaya* causes increase in RBC counts in a murine model without causing any form of toxicity. Increase in the production of RBCs may be an indication of increased bone marrow function and sufficient erythropoiesis^[8] induced by the extract. This will have to be confirmed in subsequent studies. It seems that the plant extract may contain erythropoietic property that enhances erythropoiesis as also observed in the aqueous extract of the fruits of *Solanum torvum* which increased both the number of RBCs and Hb concentration at 37.5–150 mg/kg doses in phenyl hydrazine-induced anemic rats.^[13] This observation will form the basis for the further studies on *P. pinnata*, in which anemia will be induced in the rats and the response to the treatment would be observed.

The results of the PCV, Hb concentration and RBC count as shown by the animal model would suggest that the plant extract had the potential of reversing anemia since they showed increase in blood formation.

The MCV or the mean corpuscular volume is a measure of the average RBC volume and the MCH or mean corpuscular Hb is the average mass of Hb/RBC in a sample of blood. These values increased, especially at 200 mg/kg body weight dose. This may be an indication of increase in reticulocytes (immature RBCs). This may be due to increased erythropoiesis caused by the extract. The values of MCV and MCH for the control and treated groups were higher than the physiological range in normal rats (57.00–65.00 fl and 14.60–21.30 pg, respectively).^[7] This may imply that the reticulocytes are macrocytic as expected.

Mean cell Hb concentration or mean corpuscular Hb concentration is a measure of the concentration of Hb in a given volume of packed RBCs. The values of MCHC for the treated groups showed that the RBCs were hypochromic because they were lower than the lower limit of the normal physiological range (32.00–38.50 g/100 ml)^[7] and the control.

The WBCs (leucocytes), neutrophils, lymphocytes, monocytes and eosinophils constitute the defense mechanism of the body system. The WBC count of the group treated with 50 and 400 mg/kg body weight doses exceeded the normal physiological range (5.00–8.96/mm³).^[7] Increase in the leukocyte production is always initiated either by infection, injury or toxic substance in the body.^[8] In this case, it could be due to anti-nutrients such as tannins that have been shown to be inherent in the plant extract.^[4] Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients. The percentage values for the neutrophils, lymphocytes and monocytes in the control and treatment groups were all within the normal physiological range (9.00–34.00%, 65.00–84.50% and 0.00–5.00% respectively).^[7] However, there were increases in the values for the neutrophils at the 50, 100, 200 and 800 mg/kg doses compared to the control. This result is comparable with that of *Momordica charantia* that increased the adhesion of neutrophils to nylon fibers at 900 mg/kg.^[14] Furthermore, it is similar to the observations of Vinothapooshan and Sundar.^[15] This indicated that the extract could be useful as chemotherapy for neutrophils. The apparent reduction in eosinophils in the treated rats may be to the advantage of the animals. This is an indication that the animals were not adversely affected in such a way that could elicit the response of eosinophils, that are detoxifiers in the respiratory and gastrointestinal tracts.^[8]

From the results, the maximum tolerable dose of the extract is 200 mg/kg body weight. This supports the findings from the investigation carried out on the sub-acute toxicity of

the extract on various organs in male Wistar albino rats, especially on the lungs and liver.^[4]

CONCLUSION

In this preliminary study, it was observed that repeated increasing dose treatment of the methanol extract of *P. pinnata* leaves showed that it has potentials to be used to treat anemia and therefore may improve bone marrow failure as well as serve as a therapeutic agent to increase neutrophils count. Our results, therefore, corroborated the traditional use of the leaves as an anti-anemic tonic. However, the maximum tolerable dose of the methanolic extract of the leaves of *P. pinnata* is 200 mg/kg body weight.

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