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EFFECTS OF ORAL ADMINISTRATION OF AQUEOUS EXTRACT *CAESALPINIA BENTHAMIANA* ROOTS (FABACEAE) ON HAEMATOLOGICAL PARAMETERS IN MALE *WISTAR* RATS

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ABSTRACT

Caesalpinia benthamiana (Cb) is an African tropical, particulary in Côte d'Ivoire, plant whose roots are used in traditional medicine as an aqueous decoction for many purposes, especially for erection impairment. The current study performed to evaluate the effects of aqueous roots extract of *C. benthamiana* (AECb) on haematological parameters of male Wistar rats. Twenty (20) male rats weighing between 177-186g were used for the study. The rats were divided into four groups of five each based on body weight. Group I served as the control. Groups II, III and IV were administered varying doses (25, 50 and 100 mg/kg bw respectively) of AECb. The administration was done daily for 14 days. The results showed that only AECb at 100 mg/kg bw (High dose) induced a decrease erythrocytes, haemogobin, haematocrit and thrombocytes counts compared to control group. Aqueous roots extract of *C. benthamiana* at doses of 25 and 50 mg/kg bw are nontoxic on haematological parameters studied when administered orally to 14 days in male rats.

KEYWORDS: *Caesalpinia benthamiana* roots; male Wistar rat; haematological parameters; nontoxic.

1. INTRODUCTION

Plant based medications have been employed since the dawn of civilization for prolonging the life of man and for combating various ailments. For decades the screening of medicinal

plant materials for their therapeutic values has continue to represent potential sources of new effective medicines.[1] Using plants for the treatment and cure of diseases is as old as human species itself, with popular knowledge making a great contribution to the dissemination of the therapeutic virtues of these plants. This knowledge has represented a therapeutic resource for many communities and ethnic groups.[2]

Today, the popularity of traditional medicine has greatly increased across the world in both developed and developing nations. Since 2005, The World Health Organization estimates that about 80% of the populations in developing nations use traditional medicines, most of which are plant based remedies as complementary or alternative medicine.[3] Various factors can be attributed to the increase in the use of plant based remedies. They may include perceived lower toxicity and fewer side effects of plant based medicines as these plants have been used before. To add on to the upsurge is the existence of diseases like cancer, to which no cure exists and the emergence of new diseases. The increased cases of drug resistance which are being encountered with the use of conventional medicines have favorably contributed to the use of plant based remedies.[4,5] The use of plant extracts and phyto-chemicals, both with its known toxicity effects on haematological parameters have been identified by the World Health Organization as alternative sources of therapeutic treatments.[6]

Blood is a fundamental circulatory tissue composed of erythrocytes cells, leukocytes cells and thrombocytes which are suspended in a fluid called plasma, with the important function of maintaining homeostasis. Every blood cell has a vital role in our body; for instance, leukocytes enhance the immune system and are capable to fight against infections, erythrocytes carry haemoglobin that are mainly responsible for transportation of oxygen and carbon dioxide as well as valuable in the diagnosis of anaemia; while platelets have a major role in the formation of clot. Haematological studies are essential in the diagnosis of several diseases as well as it could be a helpful tool in early sign of toxicity to cellular components of blood in response to certain natural or chemical agents.[7]

Caesalpinia benthamiana (Baill.) Herend and Zarucchi (Fabaceae), one of the traditional medicinal plant, is generally found in the tropical part of the world and situated mainly in West African countries It has been traditionally used in management of several diseases including erectile dysfunction, dysentery, urethral discharges, skin diseases and wounds.[8]

In a test carried out to determine the aphrodisiac property of the aqueous extract of the root at 50 mg/kg body weight (bw) of *C. benthamiana* (test group), the results indicate that the mounting frequency ($p < 0.001$) increased significantly while the mounting latency decreased after 30 minutes, 1.15hr and 3.15hrs of observations when compared to that of untreated rats.[9] Thus, the aqueous extract of the whole plant has been found to be none toxic to wistar rats and Swiss mice. The LD_{50} of the extract administered intraperitoneally was 1021.31 mg/kg bw. Oral administration up to 2 g/kg bw produced no deleterious effect 24 h after dosing and up to 7 days afterwards.[10] It is thus required that studies be carried out on *C. benthaminia*, aphrodisiac plant, to determine possible adverse effects in order to draw the attention of the population and health practitioners to the exposure of individuals to this drug.

Due to the shortage of research work on the effects of *C. benthamiana* roots on haematological parameters to our knowledge, this study was carried out with the aim to investigate influence of aqueous extract of *C. benthamiana* roots on haematological parameters of male albino wistar rats.

2. MATERIALS AND METHODS

2.1. Plant material

The plant material was composed of the powder roots of *C. benthamiana* (Baill) Herend and Zarucchi. Fresh roots were collected from Morefe, in the district of Yamoussoukro (Côte d'Ivoire), in August 2019. The plant was authenticated by a botanist of Nangui Abrogoua university, Abidjan, Côte d'Ivoire. The confirmation was made by the National Center of Floristic (NCF) from University Felix Houphouët Boigny (Abidjan, Côte d'Ivoire).

2.2. Animal material

Twenty (20) male albino Wistar rats weighing between 177-186g were used for this experiment. The healthy rats were bred in animal house of Physiology, Pharmacology and Pharmacopeia laboratory of the University of Nangui Abrogoua (Abidjan, Côte d'Ivoire). The rats were acclimated to temperature of 22 ± 2 °C and an alternantion of 12 hours of light and 12 hours of darkness. They were fed daily with IVOGRAIN® pellets and had access to water at will in their bottles. All procedures performed using the Organization of Economic Cooperation and Development (OECD) guideline for testing of chemicals 401[11] that were adopted by the laboratory animals of the Ethical Committee of the University (Nangui Abrogoua, Abidjan, Côte d'Ivoire)

2.3. Preparation of roots aqueous extract of *C. benthamiana*

Aqueous extract of *C. benthamiana* (AECb) was obtained according to the method described by some authors.[9,12] Fresh roots were first cleaned under a continuous stream of water for 5 min in Physiology, Pharmacology and Pharmacopeia laboratory. Then, they have cut into small pieces, dried in a oven at 50 °C for 4 days using rotary evaporator (J.P. SELECTA S, Espagne), then roughly crushed into powders with an electric grinder. The powder obtained was used for the preparation of aqueous extract.

One hundred grams (100 g) of roots powder was stirred vigorously with a magnetic stirrer for 24 hours in 1 L of cold distilled water. The mixture was subjected to double filtration through cotton wool and Whatman N°1 paper. The resulting filtrate was dried in an oven at 50 °C for 24 hours. A quantity of 11.7 g of powder with a yield of 11.7 % was obtained and stored in refrigerator at 4 °C until use. Finally, 10 g of AECb was dissolved in 20 mL of NaCl 0.9% and made up to 100 mL, to make a concentration of 100 mg/mL. This served as stock solution of the extract from where the required dosage of administration was prepared.

2.4. Experimental design and treatment of animals

A total of twenty (20) male albino Wistar rats were weighed and randomly divided into four groups of five animals each. Group I rats were given NaCl 0.9% solution (1 mL/100 kg bw) alone for 14 days and served as a control group. Groups II, III and IV were received doses of *C. benthamiana* roots extract at doses of graded doses of 25, 50 and 100 mg/kg bw/day for 14 consecutive days. The selected tested doses and duration were based upon the work of the authors.[9] The required drug was dissolved in NaCl 0.9% solution and administered orally with an oropharyngeal metal cannula.

2.5. Collection of blood sample from animals

On 15th day, blood samples were collected from each rat fasted overnight and anaesthetized by dropping each of them in a transparent jar with ether vapour. Blood was collected via retro orbital sinus of the eye and taken rapidly in anticoagulant (EDTA) containing test tube immediately used for determination of haematological indices within 24 hours of sample collection.

2.6. Haematological parameters

The samples were analysed for haematological indices using automatic hematology analyzer (MINDRAY BC 30 S). The parameters analysed include erythrocyte, leukocyte, hemoglobin,

Haematocrit, Mean corpuscular volume (MCV), Mean corpuscular Haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), thrombocytes, lymphocytes, monocytes and granulocytes (GRA) counts.

2.7. Statistical analysis

Experimental results are expressed as mean ± standard error on the mean (M ± S.E.M). The data were analysed by one-way analysis of variance (ANOVA). Tukey comparison test was used as post-hoc test. A difference was considered statistically significant when $p<0.05$. Statistical analysis and graphics of data were done using by Graph Pad Prism 5.01 (San Diego California, USA).

3. RESULTS

3.1. Effects of AECb on erythrocytic parameters

The evolution of erythrocytic parameters after oral administration of AECb at doses of 25, 50 and 100 mg/kg bw was recorded in figures 1 to 3 and table 1. Results showed significantly decrease ($p < 0.001$) erythrocyte, haemoglobin and haematocrit only in rats received AECb at 100 mg/kg bw compared to control rats for 14 days. However, AECb at doses 25 and 50 mg/kg bw did not influence ($p > 0.05$) the same parameters. Regarding MCV, MCH and MCHC values, any significant ($p > 0.05$) change observed in all groups treated with AECb at doses 25, 50 and 100 mg/kg bw when compared with the control group.

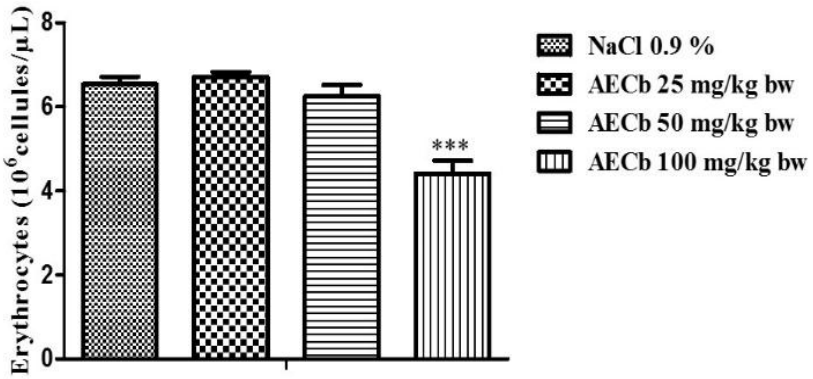

Figure 1: Effect of oral administration of aqueous extract *C. benthamiana* roots on erythrocytes for 14 days in male rats.

The values are expressed as mean ± SEM; AECb: Aqueous extract roots *C. benthamiana*; n=5 rats in each group; ***: significant difference ($p<0.001$) compared to the control group.

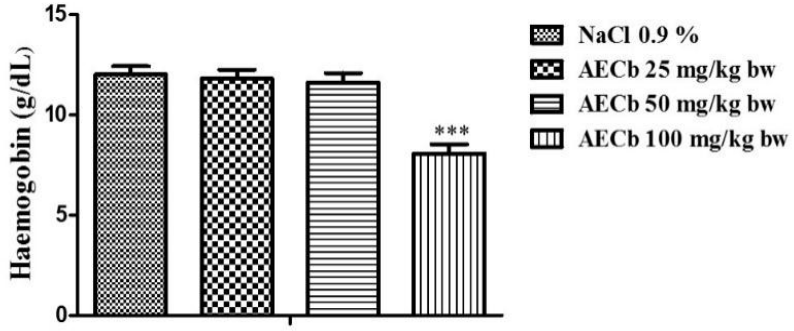

Figure 2: Effect of oral administration of aqueous extract *C. benthamiana* roots on haemoglobin for 14 days in male rats.

The values are expressed as mean ± SEM; AECb: Aqueous extract roots *C. benthamiana*; n=5 rats in each group; ***: significant difference ($p<0.001$) compared to the control group.

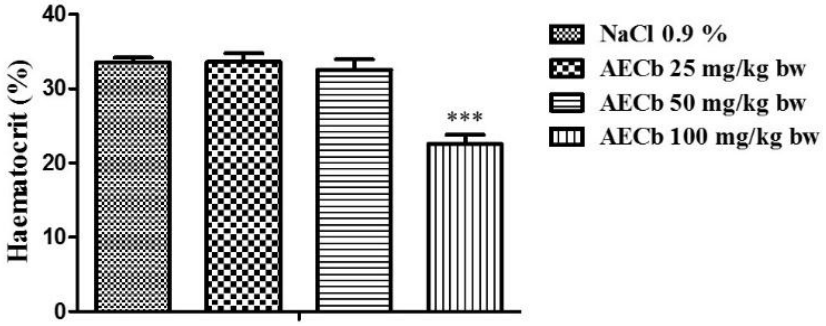

Figure 3: Effect of oral administration of aqueous extract *C. benthamiana* roots on haematocrit for 14 days in male rats.

The values are expressed as mean ± SEM; AECb: Aqueous extract roots *C. benthamiana*; n=5 rats in each group; ***: significant difference ($p<0.001$) compared to the control group.

Table 1: Effect of oral administration of aqueous extract *C. benthamiana* roots on erythrocytes indices for 14 days in male rats.

Erythrocytes indices				
	Control	AECb 25 mg/kg	AECb 25 mg/kg	AECb 25 mg/kg
MCHC (g/dL)	35.5 ± 0.96	35.4 ± 0.76	35.6 ± 0.54	36.0 ± 0.52
MCH (pg)	18.5 ± 0.77	17.4 ± 0.56	18.5 ± 0.29	18.5 ± 0.46
MCV (fL)	52.2 ±1.27	49.2 ± 0.71	52.0 ± 0.60	51.3 ± 1.07

The values are expressed as mean ± SEM; n=5 rats in each group; AECb: Aqueous extract roots *C. benthamiana*; Mean Corpuscular Volume (MCV); Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Hemoglobin Concentration (MCHC).

3.2. Effects of AECb on leukocytic parameters and thombocyte count

The aqueous extract did not induce any significant ($p > 0.05$) changes in the leukocyte, lymphocyte and monocyte counts of treated rats groups treated with AECb at doses of 25, 50 and 100 mg/kg bw compared to the control (Table 2). But, the thrombocyte count was significantly decreased ($p < 0.01$) in rats group received AECb at 100 mg/kg bw as compared to the control group (Table 2).

Table 2 : Effect of oral administration of aqueous extract *C. benthamiana* roots on leukocytic parameters and thrombocytes count for 14 days in male rats.

Parameters	Control	AECb 25 mg/kg	AECb 50 mg/kg	AECb 100 mg/kg
Leukocytes (10^6/µL)	14.18 ± 1.68	10.44 ± 0.72	11.40 ±1.05	11.12 ± 0.80
Lymphocytes (%)	9.96 ± 1.67	6.90 ± 0.76	8.30 ± 0.70	7.86 ± 0.51
Granulocytes (%)	2.94 ± 0.40	1.92 ± 0.36	2.10 ± 0.28	2.180 ± 0.43
Monocytes (%)	1.28 ± 0.22	0.84 ± 0.15	1.0 ± 0.31	1.08 ± 0.10
Thrombocytes (10^3/µL)	768 ± 47.99	775.2 ± 34.92	620.6 ± 57.74	480.6 ± 42.69**

The values are expressed as mean ± SEM; n=5 rats in each group; **: significant difference ($p<0.01$) compared to the control group; AECb: Aqueous extract roots *C. benthamiana.*

4. DISCUSSION

The effects of aqueous extract of *C. benthamiana* roots on some haematological parameters were examined in this study. The results showed that only oral administration of AECb at dose of 100 mg/kg bw (High dose) in rats decreased significantly in erythrocytes count. This was confirmed by the decreased haematocrit and haemoglobin in the same group for the period of experimentation. But, AECb at doses of 25 and 50 mg/kg bw did not influence all erythrocytic parameters. These results could indicate that the balance between the rates of production (Erythropoiesis) and destruction of red blood cells was altered by the oral administration of AECb at 100 mg/kg bw. The significant alterations of extract on these parameters could mean that the incorporation of haemoglobin into the red blood cells, the morphology and the osmotic fragility of the erythrocytes cells have been altered. These results are in agreement with those of[13] that reported significant changes in erythrocytes, haematocrit and haemoglobin counts of rats treated with aqueous extract of *Caesalpinia bonducella*, aphrodisiac plant, at 125 mg/kg bw. This extract could cause hemolytic anemia, the cause of which is linked to the possible presence of secondary metabolites with hemolyzing activity. Phytochemical studies have revealed the presence of saponins in the

aqueous extract of *C. benthamiana* roots.[14] According to[15], saponins are endowed with hemolytic property. These metabolites could weaken the membrane of red blood cells in experimental animals. In contrast, AECb at high dose did not vary in significance for MCH, MCHC and MCV.

Leukocyte cells are the first line of defense responders to pathogenic agents and other inflammatory processes. Furthermore, no significant changes were observed in leukocytes, lymphocytes, granulocytes and monocytes, which also confirmed our findings. The aqueous extract of *C. benthamiana* roots would not cause any problems on the defense cells of the organism of the normal rats. The slight variation following the administration of AECb could have been attributed to normal physiologic response of the defense mechanisms following perception of a foreign challenge.

In thrombocytes count, thrombocytopenia is a condition of abnormally low number of thrombocytes in the circulation, may result from decreased production or increased destruction of thrombocytes.[16] Some drugs provoke thrombocyte antibodies and thrombocyte destruction, resulting in thrombocytopenia[17] On the other hand, thrombocytosis is an abnormal increase in the number of circulating platelets.[18] In this study, thrombocytes count decreased significantly ($p < 0.01$) in the groups treated with AECb at the dose of 100 mg/kg bw when compared with the control group during 14 days of experimentation. This indicates that the treatment induced thrombocytopenia in normal rats. This reduction could be explained by the presence of some compounds in the extract. Some reseachers revealed the présence of flavonoids in aqueous extract of *C. benthamiana* roots.[14] They are similar to those obtained by[13] who reported that daily administration of the aqueous extract of the roots of *Caesalpinia bonducella* for 28 days at doses 31.5 and 125 mg/kg bw induced thrombocytosis in rats. In contrast, AECb at doses of 25 and 50 mg/kg bw had no influence the thrombocytes count for during the study.

5. CONCLUSION

Oral administration of aqueous extract of *C. benthamiana* roots is nontoxic on haematological parameters in male rats received with the efficace dose of 50 mg/kg bw for 14 days of experimentation. *C. benthamiana* traditionally used as an aphrodisiac plant. However, further study is required to investigate and confirms its safety in humans.

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CONFLICT OF INTEREST

All authors declare that No conflict of interest in this work.

REFERENCES

1. Omoregie ES, Osagie AU. Antioxidant Properties of Methanolic Extracts of some Nigerian Plants on Nutritionally-Stressed Rats. Nig. J. Basic Appl. Sci., 2012; 20(1): 7-20.
2. Maciel MA, Punto CA, Vieg JV. "Plantas medicinal: A recessidade de estudos multidisciphnares". Qum. Nova, 2002; 25(3): 429-438.
3. World Health Organization. Programme on Traditional Medicine. National policy on traditional medicine and regulation of herbal medicines : report of a WHO global survey. World Health Organization, 2005. https://apps.who.int/iris/handle/10665/43229.
4. Bandaranayake WM. Quality control, screening, toxicity, and regulation of herbal drugs in Modern Phytomedicine. Turning Medicinal Plants into Drugs, eds I.Ahmad, F.Aqil, and M. Owais (Weinheim: Wiley-VCH GmbH & Co. KGaA), 2006; 25-57.
5. Pan SY, Gerhard L, Si-Hua G, Shu FZ. Historical Perspective of Traditional Indigenous Medical Practices: The Current Renaissance and Conservation of Herbal Resources. EB CAM, 2014/525340.
6. Ghasemi M, Azhnaz M, Tahamtani Y. Protective effects of *Ephedra pachyclada* extract on mouse models of carbon tetrachloride-induced chronic and acute liver failure. Tissue Cell., 2014; 46(1): 78-85.
7. Etim NNA, Williams EM, Akpabio U, Offiong AEE. Haematological parameters and factors affecting their values. Agric Sci., 2014; 2(1): 37-47.
8. Dickson RA, Annan K, Komlaga G. Pharmacognostic Standardization of the Leaves and Root Bark of *Caesalpinia benthamiana*. Pharmacogn J., 2011; 2263(24): 29-36.
9. Zamble A, Martin-Nizard F, Sahpaz S, Hennebelle T, Staels B, Bordet R, Duriez P, Brunet C, Bailleul F. Vasoactivity, Antioxidant and Aphrodisiac Properties of *Caesalpinia benthamiana* Roots. J Ethnopharmacol, 2008; 116: 112-119.

10. Mbagwu HOC, Adeyemi OO. Anti-diarrhoeal activity of the aqueous extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae). J Ethnopharmacol, 2008; 116: 16-20.
11. OECD. Series on the principles of good laboratory practice and verification of compliance with these principles. ENV/MC/CHEM (98), 1998; 17: 22-23.
12. Guédé-Guina F, Vangah-Manda M, Bahi C. Potencies of MISCA, a plant source concentrate against fungi. J Ethnopharmacol, 1993; 14: 45-53.
13. Sindete M, Gbankoto A, Ganfon H, Osseni R, Yemoa A, Laleye A. Safety assessment of the ethanol extract of *Caesalpinia bonduc* (L.) Roxb. Root in Wistar rats: Acute and subacute (28-day) oral toxicity studies. Natl J Physiol Pharm Pharmacol, 2019; 9(12): 1-12.
14. Bekro YA, Mamibekrova BJA, Boua BB, Tra Bi FH, Ehilé EE. Ethnobotanical study and phytochemical screening of *Caesalpinia benthamiana* (Baill.) Herend. and Zarucchi (Caesalpiniaceae). Sci. Nat, 2007; 4(2): 217-225.
15. Bissinger R, Modicano P, Alzoubi K, Honisch S, Faggio C, Abed M, Lang F. Effect of saponin on erythrocytes. Int. J. Hematol, 2014; 100: 51-59.
16. Tousson E, El-Moghazy M, El-Atrsh E. The possible effect of diets containing Nigella sativa and *Thymus vulgaris* on blood parameters and some organs structure in rabbit. Toxicol Ind Health, 2011; 27: 107-116.
17. Weingand K, Brown G, Hall R, Davies D, Gossett K, et al. Harmonization of animal clinical pathology testing in toxicity and safety studies. The Joint Scientific Committee for International Harmonization of Clinical Pathology Testing. Fundam Appl Toxicol, 1996; 29: 198-201.
18. Aajibade TO, Olayemia FO, Arowolo ROA. The haematological and biochemical effects of methanol extract of the seeds of *Moringa oleifera* in rats. J Medic Plants Res., 2012; 6: 615-621.