

***In vivo* action on the hemogram of *Oryctolagus cuniculus* of *Mucuna pruriens* and *Millettia pinnata* (Fabaceae) minerals.**

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Authors' contributions

All the authors mentioned have each contributed at their own level to the realization of the work. OCO defined the theme of the work, analyzed and interpreted the manuscript. MHA and KBA provided the venom and guided the work. GCB created the conditions for animal husbandry, collected blood samples from the animals, performed the analyses of the various parameters and wrote the manuscript. AJD provided scientific support for the work.

ABSTRACT

Aims: Evaluate the *in vivo* activity of minerals from two plants on hematological parameters.

Place and Duration of Study: Department of Biochemistry-Microbiology and at the laboratory of Daloa Hospital (Côte d'Ivoire), between December 2019 and April 2020.

Methodology: During this study, twenty-seven rabbits, seventeen males and ten females were used. For its realization, two portions were carried out (experimental and control). The experimental portion consisted of seven batches of three rabbits (two males and one female), while the control portion consisted of two batches of three males and three females for each batch. Each rabbit of the two portions was collected separately in a purple tube (EDTA) with the only difference that for the experimental portion, a given rabbit was first scarified and then a previously prepared precise potion (P) was separately applied to the scarified area of the rabbit and blood was collected from day 4 onwards for analysis of hematological parameters.

Results: This study reveals that:

- P1 (ashes of *Mucuna pruriens*, *Millettia pinnata* and viper skull) and P3 (ashes of *Millettia pinnata*) have a stimulating activity on hematopoiesis.

- P1, P3, P4 (ashes of viper skull), P6 (ashes of viper skull and *Mucuna pruriens*) and P7 (ashes of viper skull and *Millettia pinnata*) have a stimulating action on the synthesis of hemoglobin.

- P1, P2, P3, P5 (ashes of *Mucuna pruriens* and *Millettia pinnata*), P6 and P7 induce hyperlymphocytosis in some animals; whereas P1, P3 and P6 promote hyperleukocytosis in each female of lots 3; 5 and 8.

- P2 (*Mucuna pruriens* ash), P3, P5, P6 and P7 induce thrombocytosis in females of the various lots; while P1, P2, P4, P6 and P7 promote thrombocytopenia in some animals.

Conclusion: Minerals from the two plants have different effects on rabbit hematological parameters.

Keywords: Minerals, *Mucuna pruriens*, *Millettia pinnata*, hemogram

1. INTRODUCTION

Envenomation is the introduction into the body of a toxic substance, venom, due to the bite of a snake, the sting of a scorpion, a wasp, etc.. The most dangerous is that caused by snake bites [2, 11]. Indeed, snake venom contains a complex mixture of enzymes, peptides, and proteins of low relative molecular weight, with specific chemical and biological activities that can lead to death from neurological and/or hematological disorders [7, 11]. In Africa, generally speaking, the venomous snakes responsible for all these disasters are mainly Viperidae and Elapidae. Viperidae (vipers) are the most widespread venomous species and do the most damage, whereas Elapidae (spitting cobra and mamba) are the most dangerous snakes because of the high toxicity of their venom [13]. It is classic to oppose cobraic poisoning, essentially neurotoxic, and viperine poisoning, dominated by necrosis and hemorrhagic syndromes. In practice, this distinction must be qualified [6]. In effect, the diversity of substances, contained in the venoms of the species of these two families of snakes, vary according to the species and even between individuals of the same species, which makes certain species exceptional by the action of their venom. Among these exceptions is the venom of *Naja nigricollis* (spitting cobra), one of the most dangerous and representative species of Elapidae in Africa. Its venom contains cytotoxins that target certain blood cells and those of certain organs such as the heart. Its action on these organs creates dysfunctions whose effects associated with those of these neurotoxins could have a serious impact on the victim's breathing and lead to his death [9, 5].

In reality, the haematological parameters or haemogram are made up of blood figurative elements (blood cells) divided into red lineage cells (red blood cells, reticulocytes), white lineage cells (lymphocytes, granulocytes, monocytes) and platelets. Under physiological conditions, the proportion of each cell type is within a range determined for each species. The absolute or relative quantity of different cell types may vary following exposure to a toxicant or under pathological conditions [8]. The same author defines hematological toxicity as an increase or decrease in the number of peripheral blood cells of one or more cell lines. These cytopenias have various consequences: anemic syndrome, hemorrhagic, infectious by immunosuppression, etc.... The origin of this change in the blood count may be central by spinal cord injury or peripheral by immune destruction or not. According to [14], certain natural bioactive substances can also have an effect on white blood cells and haemoglobin.

In actual fact, according to the World Health Organization in 2013, approximately 80% of the populations of developing countries will be using traditional medicine and in particular herbal medicine for their health care needs. Effectively, the African floristic heritage is very rich in medicinal plants whose effectiveness is proven. The latter abounds in nearly 5,000 medicinal species [1, 17]. In West Africa, particularly in Benin, 80% of snake bites are reported to use traditional treatment rather than modern Western medicine [4, 10]. In Côte d'Ivoire, some researchers report that the roots of *Securidaca longepedunculata* (Polygalaceae) can be used in the case of Elapidae poisoning [12, 18].

Also in Côte d'Ivoire, specifically in the Bouaké region, some traditional practitioners use *Millettia pinnata* and *Mucuna pruriens*, two species of the Fabaceae family, for cases of envenimation.

It is with the aim of rationally exploiting this heritage, to provide a scientific basis for the use of these plants and to contribute to the discovery of new drug leads that this study was carried out. Its objective was to find out the in vivo effect of *Millettia pinnata* and *Mucuna pruriens* on the haemogram of rabbits.

2. MATERIALS AND METHODS

2.1 Plant material

The plant material is composed of *Mucuna pruriens* and *Millettia pinnata*. They were harvested in December 2019 in Bouaké (Central Côte d'Ivoire).

2.2 Animal material

For this study, twenty-seven rabbits including seventeen males and ten females of Hyplus breed, aged two and a half months, were purchased from a breeder in the locality of Daloa (Côte d'Ivoire). After the acclimatization period of about three weeks, the weight of the rabbits varied between 1.45 and 2.4 kg. Beside this animal model, the viper skulls were provided by a medico-druggist.

2.3 Method of preparation of minerals

For its realization the various plants were harvested in Bouaké, washed, cut then dried in the shelter of the sun, at room temperature during one week. The plant organs were then dried in an oven at a temperature of 70 °C for three days. After this drying time, the organs (plant and animal) obtained were incinerated in a muffle furnace for 13 hours at 550 °C. The resulting ashes were weighed using a precision balance. They are unctuous with the exception of the viper skull which is rough. The colors vary from gray to brown.

The combination of ashes from the various organic products resulted in the following potions:

- P1 consists of the ashes of the two plants and the skull of a viper;
- P2, P3 and P4 are respectively and only made up of ashes of *Mucuna pruriens*, *Millettia pinnata* and the skull of viper;
- P5 is composed of the ashes of *Mucuna pruriens* and *Millettia pinnata*;
- P6 consists of the ashes of the skull of viper and *Mucuna pruriens*;
- P7 is constituted by the ashes of the skull of viper and *Millettia pinnata*.

2.3.1 Calculation of incineration efficiency

The formula below was used to calculate the weight of dry matter of the organs used.

$$Ac = \frac{\text{Mass of ashes}}{\text{Dry matter}} \times 100$$

Ac : Ash content

2.3.2 Scarification method for experimental batches

To scarify the experimental batches, the following potions:

- P1 was used for lots 3;
- P2 has been used for lot 4;
- P3 has been used for lots 5;
- P4 served for lot 6;
- P5 is used for lot 7;
- P6 is used for Lot 8;
- P7 has been used for lot 9.

Each experimental batch consisted of two males and one female. However, before scarification, the affected areas (toes of the left paw and tarsus of the right paw) were exposed with a pair of scissors. Then, a separate quantity of 0.45 mg of the potion previously prepared was applied to each affected area of each given batch. Experimental testing began four days after scarification.

2.4 Method of blood collection

In general, blood samples were taken from the short saphenous vein and/or the femoral vein. The restraint method was performed by three people. The areas where these veins were located were previously exposed with a pair of scissors. The vacutainers into which the needles were inserted allowed the sampling to be carried out using the purple tubes (EDTA). The resulting tubes were stored in a cooler containing ice and then transported to the laboratory for analysis.

3. RESULTS

3.1 Results of control rabbit blood counts

The results of the control rabbit blood counts (lot 1 and lot 2) in Table 1 indicate that the reference value:

- of white blood cells is between 10.31 and $11.23 \times 10^3/\text{mm}^3$ for males and between 4.88 and $6.86 \times 10^3/\text{mm}^3$ for females;
- red blood cells are included in the range of 5.62 to $6.62 \times 10^6/\text{mm}^3$ for males and 5.86 to $6.10 \times 10^6/\text{mm}^3$ for females;
- hemoglobins is 11.32 and 12.15 g/dL for males and 6.39 and 12.37 g/dL for females;
- of hematocrit is from 34.63 to 39.31 %, and 36.07 to 38.07 % for males and females respectively;
- of MGVs is situated between 59.50 - $61.64 \mu^3$ and 61.91 - $64.75 \mu^3$ for males and females respectively;
- of the MCHC is comprised between 18.35-20.05 pg/L for males and 18.62-19.32 pg/L for females;
- of MCHs is within the range of 30.8-32.6 % and 29.10-30.37 % for males and females, respectively;
- of platelets is included between 401.83 - $770.83 \times 10^3/\text{mm}^3$ for males and 263.82 - $276.84 \times 10^3/\text{mm}^3$ for females;
- of lymphocytes is 44.59 and 57.61%, and 35.19 and 36.41% for males and females, respectively.

Table 1: Hemogram of control rabbit batches

	Lot 1: Males controls						Lot 2: Females controls					
	M7	M10	M16	Ave	Sd	Reference values	F1	F10	F12	Ave	Sd	Reference values
Weight	1.9	2.4	1.5	1.92	0.48	1.44-2.4	1.7	2	2	1.9	0.17	1.73-2.07
WBC	11	11	11	10.77	0.46	10.31-11.23	7	5.2	5.4	5.87	0.99	4.88-6.86
RBC	5.7	6	6.7	6.12	0.50	5.62-6.62	6.1	5.9	5.9	5.98	0.12	5.86-6.10
Hg	11	12	12	11.73	0.42	11.32-12.15	11	11	5.9	9.38	2.99	6.39-12.37
Ht	35	37	40	36.97	2.34	34.63-39.31	37	36	38	37.07	1.00	36.07-38.07
MGV	62	61	59	60.57	1.07	59.50-61.64	62	64	64	63.33	1.42	61.91-64.75
MCHC	20	19	18	19.2	0.85	18.35-20.05	19	19	19	18.97	0.35	18.62-19.32
MCH	33	32	31	31.7	0.9	30.8-32.6	30	29	30	29.73	0.64	29.09-30.37
Pt	769	590	400	586.33	184.5	401.83-770.83	264	270	277	270.33	6.51	263.82-276.84
LC%	45	50	58	51.1	6.51	44.59-57.61	36	35	36	35.8	0.61	35.19-36.41

RBC = Red Blood Cells ($\times 10^6/\text{mm}^3$); **WBC** = White Blood Cells ($\times 10^3/\text{mm}^3$); **Hg** = Hémoglobine (g/dL); **Ht** = Hématocrite (%); **MGV** = Mean Global Volume (μ^3); **MCHC** = Mean Corpuscular Hemoglobin Content (pg/L); **MCH** = Corpuscular (or Globular) Mean Hemoglobin Concentration (%); **Pt** = Platelets ($\times 10^3/\text{mm}^3$); **LCva** = Lymphocytes in absolute value (%); **M**= Male; **F**= female; **Ave** = Average; **Sd** = Standard deviation

3.2 Results of the haemogram of rabbits scarified as a preventive measure

The analysis of the haemogram of rabbits scarified as a preventive measure before envenomization in Table 2 shows:

- an increase in both red blood cells (RBC), hemoglobin (Hg) and hematocrit (Ht) compared to a decrease in lymphocytes (LC) in the two male rabbits from lot 3; while the mean corpuscular (or globular) hemoglobin concentration (MCH) and mean globular volume (MGV) are increased separately in every other male rabbit. For the female of the lot, white blood cells, mean corpuscular hemoglobin content (MCHC), MCH and lymphocytes are elevated whereas platelets (Pt) are decreased relative to their respective standards.
- an elevation of both hematocrit in both lot 4 males while there was a reduction in both white blood cells (WBCs) and platelets and only a decrease in lymphocytes in one out of two males, and an increase in both WBCs and hemoglobin in one out of two males. In the female of the same lot, there is an elevation of both MCHC, MHC, platelets and lymphocytes.
- in males of lot 5, a simultaneous increase in RBC, MGV and LC of both males, an increase in Hg, Ht, MCHC and MHC of one male, and a decrease in both WBC of both males. For the female of the same lot, there is an increase in WBC, MCHC, MHC, Pt and LC.
- at lot 6, there is a concomitant increase in Hg, MCHC and MHC of both males, and also Ht and MGV of one male; there is a decrease in LC of both males. As for the female of the lot, MCHC, MHC and LCs are elevated; while Ht, MGV and Pt are reduced.
- an elevation of the MGV of both males and the female, and only the MCHC, Pt and LCs of the female in lot 7. A decrease in WBC and Hg of both males, RBC of all individuals and LC of a male from the same lot.
- an increase in Hg of both males, WBC, MGV, MHC and LC of one male and female, Ht and MCHC of one male and Pt of the female of lot 8. The decrease in lot 8 is observed in WBC, Pt and LC of a male and RBC of the female.
- at the level of lot 9, there is an elevation of MGVs of both males, RBC and Hg of one male, MHC and LC of one male and female, and MCHC and Pt of the female. In the same lot, both males show a reduction in Pt; in one male, WBC and RBC decrease; in one male and the female, Ht declines; while in the female, MGV decreases.

Table 2: Hemogram of rabbits scarified as a preventive measure before envenimation

	Lot 3			Lot 4			Lot 5			Lot 6			Lot 7			Lot 8			Lot 9		
	M3	M13	F3	M14	M9	F11	M8	M11	F7	M1	M6	F6	M5	M12	F14	M15	M2	F13	M7	M4	F2
Weigh	2.35	1.7	1.8	1.8	2.1	1.8	1.8	1.9	2	1.9	1.9	1.9	1.7	2.3	1.7	1.9	1.9	2	1.9	1.9	1.9
WBC	10.5	10	7.2	14	9.6	5.4	8.8	7.7	8.2	11	11	6	6.3	5.2	6.8	13	9.6	7.4	9.6	11	5.4
RBC	6.77	6.9	5.9	6.6	5.6	5.8	6.8	7	5.8	6	6,6	5.6	5.5	5.3	5.6	6.3	5.6	5.6	5.6	7.1	5.8
Hg	13.1	14	12	13	11	11	14	12	11	13	14	11	11	10	11	13	13	12	11	14	11
Ht	40	43	37	40	40	35	43	38	36	39	41	33.4	35	33	36.6	39	42	37	36	14	11
MGV	59	63	62	61	60	60	63	65	62	60	62	60	64	63	66	62	61	66	63	62	60
MCHC	19.3	20	20	19	19	20	21	20	20	21	21	20	20	19	20	20	19	21	20	20	20
MCH	32.7	31	32	31	31	33	33	31	32	33	33	33	31	31	30	33	32	32	32	33	33
Pt	460	692	234	709	340	412	735	455	461	401	416	108	694	441	321	728	338	457	346	303	412
LC%	34.1	36	63	15	54	60	70	66	54	34	35	64	35	59	77.6	36	58	53	56	64	60

RBC = Red Blood Cells ($\times 10^6/\text{mm}^3$); **WBC** = White Blood Cells ($\times 10^3/\text{mm}^3$); **Hg** = Hémoglobine (g/dL); **Ht** = Hématocrite (%); **MGV** = Mean Global Volume (μ^3); **MCHC** = Mean Corpuscular Hemoglobin Content (pg/L); **MCH** = Corpuscular (or Globular) Mean Hemoglobin Concentration (%); **Pt** = Platelets ($\times 10^3/\text{mm}^3$); **LCva** = Lymphocytes in absolute value (%); **M**= Male; **F**= female; Light gray: Low value; Dark gray and bold: High value.

4. DISCUSSION

4.1 Results of control rabbit blood counts

The results of this study reveal that with the exception of the white blood cell and platelet baseline values which are elevated in males and females, the other baseline values are in approximately the same ranges. In addition, the set of hematological baseline values obtained in this study is generally consistent with those of [16], with the exception of male MGVs below and MCHCs above those of [16].

The results of the haemogram of rabbits treated as a preventive measure, prior to venom injection, showed in general an increase in both red blood cells, haemoglobin and haematocrit values in the two male rabbits of lot 3 and one rabbit of lot 5 treated respectively with P1 and P3. According to [15, 3], in contrast to anemia, polyglobulia corresponds to an increase in total blood cell mass, which will be suspected when there is a proportional increase in hemoglobin and hematocrit values. It is therefore possible to say that these potions (P1 consisting of the ashes of the two plants and the skull of a viper and P3 consisting only of ashes of *Millettia pinnata*) would have a stimulating activity of hematopoiesis. Thus, potion P3 (ashes of *Millettia pinnata*), whose effect caused the elevation of both red blood cell and MGV values in one rabbit from lot 5 and two rabbits from lot 3, would have this stimulating activity on hematopoiesis and would thus confer it to potion P1 (ashes of the two plants and of the viper's skull) of which it is a part. According to [8], hematopoiesis is physiologically limited in adulthood to the medullary cavity of the axial skeletal bones and the epiphysis of long bones, but in the event of an increase in the demand for cells, a hematopoiesis qualified as extramedullary can extend to the liver, the spleen and the lymph nodes.

4.2 Results of the haemogram of rabbits scarified as a preventive measure

Table 2 shows simultaneous elevation of hemoglobin and MHC values in one rabbit from lot 3; one rabbit from lot 5; two rabbits from lot 6, one rabbit from lot 8, and one rabbit from lot 9. These animals were treated respectively with P1 (ashes from both plants and viper skull); P3 (ashes from *Millettia pinnata*); P4 (ashes from viper skull); P6 (ashes from viper skull and *Mucuna pruriens*) and P7 (ashes from viper skull and *Millettia pinnata*). According to [15], hemoglobin synthesis occurs in the erythroblast and at a certain high MHC value, this synthesis can be stopped. Thus, it could be said that these potions (P1, P3, P4, P6 and P7) may have a stimulating action on hemoglobin synthesis. Insofar as P3 and P4 are composed solely and respectively of *Millettia pinnata* ash and viper skull ash; then these enter at the same time into the constitution of P1 and P7; while the viper skull ash is essentially in P6, it is also possible to say that the stimulating action of these various aforementioned potions is due to the presence of the ashes of the two organic products (*Millettia pinnata* and viper skull) in them. However, P3 (ash from *Millettia pinnata*), P4 (ash from viper skull) and P7 (ash from

viper skull and *Millettia pinnata*) would have more stimulating action for the synthesis of hemoglobin. In the same table, an increase in lymphocytes was observed in one rabbit from lots 3; 4 and 7; two rabbits from lots 8 and 9 and three rabbits from lot 5 with simultaneous elevation of white blood cells in each female from lots 3; 5 and 8. The above-mentioned lots were treated respectively with P1 (ashes of both plants and viper skull), P2 (ashes of *Mucuna pruriens*), P5 (ashes of *Mucuna pruriens* and *Millettia pinnata*), P6 (ashes of viper skull and *Mucuna pruriens*), P7 (ashes of viper skull and *Millettia pinnata*) and P3 (ashes of *Millettia pinnata*). [15] suggest that an increase in white blood cells indicates hyperleukocytosis; while an increase in lymphocytes indicates hyperlymphocytosis. Hyperlymphocytosis is involved in cellular and humoral immunity, i.e. in the production of antibodies. So, some animals treated with P1; P2; P3; P5; P6 and P7 obey to hyperlymphocytosis; whereas each female of lots 3; 5 and 8 has hyperleukocytosis and this would be due to the solicitation respectively of P1, P3 and P6 for their care. At the platelet level, an increase was observed for the female of lots 4; 5; 7; 8 and 9 treated respectively with P2 (ashes of *Mucuna pruriens*), P3 (ashes of *Millettia pinnata*), P5 (ashes of *Mucuna pruriens* and *Millettia pinnata*), P6 (ashes of viper skull and *Mucuna pruriens*) and P7 (ashes of viper skull and *Millettia pinnata*). These different observations would indicate thrombocytosis (or hyperplaquettosis) according to [15]. Consequently, the action of the different ashes (P2, P3, P5, P6 and P7) solicited on the various batches mentioned above is thrombocytosis. According to the same author, the decrease in platelets in the female of lot 3 treated with P1, in the male of lot 4 cared for with P2, in the female of lot 6 treated with P4, in the male of lot 8 cared for with P6 and in the two males of lot 9 given P7 would show thrombocytopenia. Thus, the various ashes (P1, P2, P4, P6 and P7) used would have this thrombocytopenia in the different animals mentioned above.

5. CONCLUSION

The results of this study reveal that:

- P1 and P3 would have hematopoiesis-stimulating activity. Nevertheless, P3, whose effect caused the elevation of both red blood cell and MGV values in two rabbits of lot 5 and one rabbit of lot 3, would have this stimulating activity on hematopoiesis and would thus confer it to the P1 potion of which it is a part.
- P1, P3, P4, P6 and P7 have a stimulating action on hemoglobin synthesis. As far as P3 and P4 are composed solely and respectively of *Millettia pinnata* ash and viper skull ash; then these enter at the same time in the constitution of P1 and P7; while the viper skull ash is essentially in P6, it is also possible to say that the stimulating action of these various above-mentioned potions is due to the presence of the ashes of the two organic products (*Millettia pinnata* and viper skull) in them. But P3, P4 and P7 would have more stimulating action for the synthesis of hemoglobin.
- P1, P2, P3, P5, P6 and P7 induce hyperlymphocytosis in some animals; whereas P1, P3 and P6 promote hyperleukocytosis in each female of lots 3; 5 and 8.
- P2, P3, P5, P6 and P7 induce thrombocytosis in females of the various lots; whereas P1, P2, P4, P6 and P7 promote thrombocytopenia in some animals.

So, the minerals from the two plants have different effects on the hematological parameters of the rabbit.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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