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In Vivo Antivenom Action of *Mucuna pruriens* and *Millettia pinnata* (Fabaceae) Minerals on the Blood Count of Oryctolagus Cuniculus

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ABSTRACT

The objective of this study was to evaluate the antivenom action of minerals from two plants on the hematological parameters of rabbits. To carry out the study, thirty-six rabbits (nineteen males and seventeen females) were divided into three portions (E1, E2 and E3). For E1, each rabbit from the control and experimental lots was sampled separately. However, the experimental rabbits were collected 20-30 minutes after envenomation with *Naja nigricollis*. In the case of E2, each rabbit in the experimental batch was first separately scarified with the prepared potions and then distinctly harvested the following day. Then, every individual was individually envenomated and then uniquely collected. In E3, the animal was independently envenomed, scalped with the potions and then sampled three days later. All blood samples were taken in various purple tubes and then transported to a cooler for determination of hematological parameters. The results revealed that the use: - As a preventive measure of *Millettia pinnata* without envenomation, has an essentially stimulatory action on hematopoiesis and hemoglobin synthesis, and induces hyperlymphocytosis in all individuals. - As a preventive measure of *Millettia pinnata* or *Mucuna pruriens* followed by envenomation has shown a variability of action.- As a curative measure of *Millettia pinnata* after envenomation causes the variation and normalization of many hematological parameters.Thus, for the development of phytomedicines, it would be advisable to seek the minerals from *Millettia pinnata* against ophidian envenomations.

INTRODUCTION

Envenomation is the introduction into the body of a toxic substance, the 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, 17]. Indeed, the venom contains a complex mixture of enzymes, peptides and proteins of low relative molecular weight, with specific chemical and biological activities, which can lead to death through neurological and/or hematological disorders [13, 17]. This envenomation is a problem affecting the five continents of the world. Worldwide, the annual incidence of these snakebites exceeds six million [4, 11, 9]. However, Africa records more than one million bites causing 600,000 cases of envenomation. Also in Africa, nearly 250,000 patients are treated, nevertheless, there are more than 20,000 deaths [7, 8].

In Africa, in general, the venomous snakes responsible for all these disasters are mostly Viperidae and Elapidae. The Viperidae (vipers) are the most widespread of the venomous species and do the most damage while the Elapidae (naja, mamba) are the most dangerous of the snakes because of the high degree of toxicity of their venom [20]. It is traditional to oppose cobraic envenomations, essentially neurotoxic, and viperine envenomations, dominated by necrosis and hemorrhagic syndromes. In practice, this distinction must be qualified [8]. Indeed, 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 in 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 cytoxins 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 breathing of the victim and lead to his death [15, 6].

Given the diversity and severity of the consequences of snakebites, various means of treatment are used, depending on the geographical situation, the beliefs of the populations, the cost, the precocity and the specificity [10]. These various means of treatment involve two types of medicine. Modern medicine, whose principle of treatment is based on the administration of anti-venom serum as soon as possible, is considered too specific, more expensive, less preservable and less accessible, but whose effectiveness has been scientifically proven. As for traditional medicine, it uses plants or other natural substances

that are more accessible, preservable and effective according to a belief generally based on traditional practitioners and testimonies from a large number of patients [12].

According to the World Health Organization in 2013, about 80% of the populations of developing countries use traditional medicine and in particular herbal medicine for their health care needs. The African floral heritage is very rich in medicinal plants with proven effectiveness. It is full of nearly 5000 medicinal species [1, 22].

In West Africa, particularly in Benin, 80% of people bitten by snakes report that they use traditional treatment rather than modern Western medicine [5, 16]. In Côte d'Ivoire, some researchers report that the roots of *Securidaca longepedunculata* (Polygalaceae) can be used in cases of envenomations by Elapidae [19, 25]. Still in Côte d'Ivoire, precisely in the region of Bouaké, some traditional practitioners use *Millettia pinnatta* and *Mucuna pruriens*, two species of the Fabaceae family, for cases of envenomations.

It is in the concern to exploit rationally this heritage, to give a scientific basis to the use of these plants and to bring its contribution to the discovery of new heads of series of drugs that this present study was led.

MATERIALS AND METHODS

MATERIALS

Biological materials

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

Animal material

For this study, thirty-six (36) rabbits of which nineteen (19) males and seventeen (17) females of the Hyplus breed, aged two (2) months and a half, were purchased from a breeder in the locality of Daloa (Côte d'Ivoire). After the acclimatization period, the weight of the rabbits varied between 1.45 and 2.4 kg. Besides this animal model, viper skulls and *Naja nigricollis* venom were also used. The viper skulls were provided by a medicodrugist while the *Naja nigricollis* venom was provided by the Pasteur Institute of Adiopodoumé (Côte d'Ivoire).

METHODS

Method of preparation of minerals

For its realization, the various plants were harvested in Bouaké, washed, cut up and then dried in the sun at room temperature for one week. Then, the plant organs were 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 ashes obtained were weighed with a precision balance. They are smooth except for the viper skull which is rough. The colors vary from gray to brown.

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

- P1 made up of the ashes of the two plants and the skull of viper;

- P2, P3 and P4 are constituted respectively and only of ash of *Mucuna pruriens*, *Millettia pinnata* and skull of viper;

- P5 is composed of the ashes of *Mucuna pruriens* and *Millettia pinnata*;

- P6 is formed by the ashes of the skull of viper and *Mucuna pruriens*;

- P7 consists of the ashes of viper skull and *Millettia pinnata*.

Calculation of incineration efficiency

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

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

Tc: Ash content

Method of scarification of experimental batches

To scarify the experimental batches, the following potions:

- P1 was used for batch 2;

- P2 served for batch 3;

- P3 has been used for batch 4;

- P4 used for lot 5;

- P5 was used in batch 6;

- P6 served for lot 7;

- P7 was used for batch 8.

Every experimental batch consisted of two (2) males and one (1) female. However, prior to scarification, the affected areas (toes of the left paw and tarsus of the right paw) were bared with a pair of scissors. Thereafter, a separate amount of 0.45 mg of the previously prepared potion was applied to each affected area of each given batch. The experimental tests continued for three days after the scarification (for the preventive test) and the curative test.

Blood collection method

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 are located were previously exposed with a pair of scissors. The vacutainers in which the needles were inserted were used to collect the samples using purple tubes (EDTA). The tubes obtained were kept in a cooler containing ice and then transported to the laboratory for analysis.

***In vitro* hemolysis test method**

To perform this test, two (2) control batches (batch 1 and batch 2) were constituted. Lot 1 consisted of three (3) males and lot 2 of three (3) females. Among these control batches, a blood sample was taken from one of them in order to perform the *in vitro* hemolysis test of *Naja nigricollis* venom. To realize this test, ten (10) tubes were used including a control tube and nine (9) experimental tubes. The stock solution was prepared in tube 1 by dissolving a quantity of 1.6 mg of venom crystals in 1 mL of physiological water. In the remaining 9 tubes (tubes 2 to 10), a volume of 0.5 mL of physiological water was added. The venom concentration ranges were prepared using the double dilution technique of geometric reason 1/2. It consisted in taking 0.5 mL of the stock solution (tube 1) and transferring it to 0.5 mL of physiological water in tube 2 and then homogenizing it. This procedure was repeated until tube 9. From tube 9, a volume of 0.5 mL was taken and subsequently discarded. Therefore, the concentrations varied in the tubes from 1.6 mg/mL to 6.26.10-3 mg/mL. To these 9 experimental tubes and to the control tube (tube 10), 5 drops of rabbit whole blood were added, which was manually homogenized. After homogenization, all the preparations were

incubated at room temperature for 30 to 40 minutes for microscopic observation. This observation was performed at magnification 40 (X40). For this purpose, the preparations of tube 1 (SM); 2; 3 and tube 10 (control tube) were diluted separately to 1/5th. Subsequently, each dilution was spread between slide and coverslip by putting the dilution of tube 10 (control) and an experimental dilution (for example tube 1). This last operation was also done for tube 2 and tube 3.

Method of carrying out the experimental tests

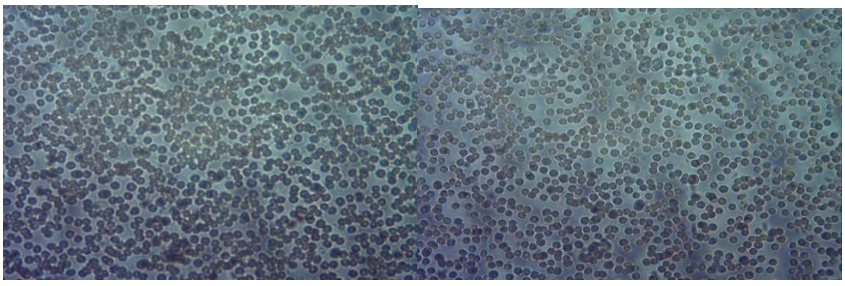
For the experimental tests, 2 mg of venom crystals were dissolved in 0.5 mL of physiological solution to obtain a concentration of 4 mg/mL. This is the concentration that was injected into the rabbits. According to [12], the median lethal dose for a 2 kg rabbit is 2 mg/kg of body weight by intra-muscular injection.

RESULTS AND DISCUSSION

RESULTS

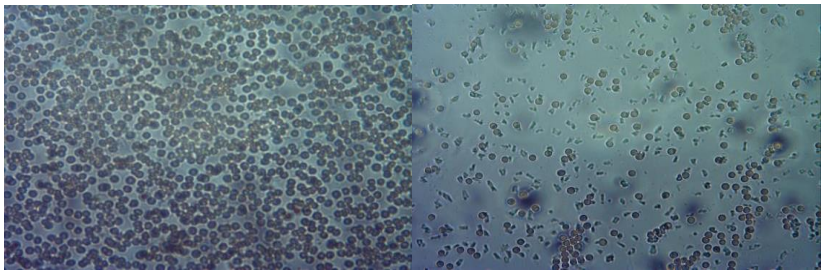
Result of the hemolytic power of the venom *in vitro*

Figures 1 (A and B); 2 (A and B) and 3 (A and B) are the results of the effects of different concentrations of venom tested *in vitro* on red blood cells. These figures show that in general, the density of red blood cells varies compared to the control according to the concentrations tested. However, this density is higher when the concentration is low (0.4 mg/mL) and low when the concentration is high (1.6 mg/mL).



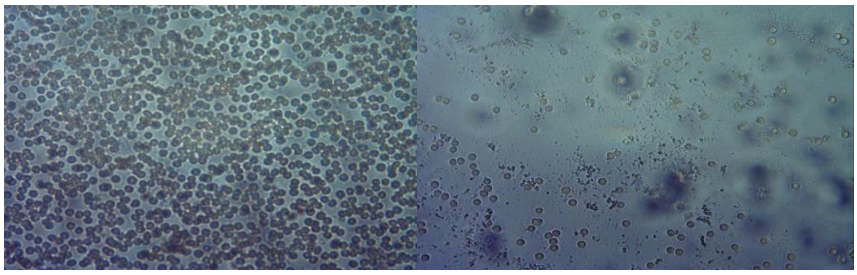
A B

Figure No. 1: Hemolysing power of venom on red blood cells (at magnification 40)
1. A: control red blood cells; 1. B: red blood cells at 0.4 mg/mL venom concentration



A B

Figure No. 2: Hemolysing power of venom on red blood cells (at magnification 40) 2. A: control red blood cells; 2. B: red blood cells at 0.8 mg/mL venom concentration



A B

Figure No. 3: Hemolysing power of venom on red blood cells (at magnification 40) 3. A: control red blood cells; 3. B: red blood cells at a concentration of 1.6 mg/mL venom

Results of blood counts of control rabbits, untreated envenomed rabbits and preventively scarified rabbits

The results of the haemogram of the control rabbits (batch 11 and 12) in Table 1 indicate that the reference value:

- Of white blood cells (WBC) is included between 10.30 and 11.23.10^3/mm^3 for males and between 4.88 and 6.85.10^3/mm^3 for females;

- Of red blood cells (RBC) is comprised between 5.62 and 6.62.10^6/mm^3 for males and 5.86 and 6.10.10^6/mm^3 for females;

- Hemoglobin (Hg) is 11.32 and 12.15 g/dL for males, and 6.39 and 12.36 g/dL for females;

- Hematocrit (Ht) is between 34.63 and 39.30%, and 36.06 and 38.07% for males and females respectively;

- Mean hemoglobin cell volume (MHCV) is in the range of 59.50-61.64 μ^3 and 61.91-64.76 μ^3 for males and females respectively;

- Mean corpuscular hemoglobin levels (MCHL) is within 18.35-20.05 pg/L for males and 18.62-19.32 pg/L for females;

- Of mean corpuscular hemoglobin concentrations (MCHC) is included between 30.8-32.6% and 29.10-30.37% for males and females respectively;

- Platelets (Pt) are found between 401.83-770.83.10^3/mm^3 for males and 263.83-276.84.10^3/mm^3 for females;

- Lymphocytes (LC) are 44.59 and 57.61%, and 35.19 and 36.41% for males and females respectively.

The blood counts of the envenomed and untreated rabbits of batch 1 in Table 1 allowed us to observe in comparison with the reference values of each parameter studied and according to sex that:

- In terms of males, there are:

- a low WBC value in both males,

- a small value of RBC in both males,

- a weak value of Hg in both males,

- a decrease in Ht in one male,

- an increase in MHCV in both males.

- At the female level, there are:

- a reduction in RBCs,

- an elevation of MHCV, MCHL, Pt and LC.

All animals in this experimental batch were found dead the next day.

Blood count analysis of rabbits scarified preventively before envenomation in Table 2 shows:

- All animals in the various lots were found alive four days after scarification.

- An increase in both red blood cells (RBC), hemoglobin (Hg) and hematocrit (Ht) whereas there is a decrease in lymphocytes (LC) in both batch 2 male rabbits; while mean corpuscular (or globular) hemoglobin concentration (MCHC) and mean corpuscular volume (MHCV) are separately elevated in one male out of two, platelets (Pt) and mean corpuscular hemoglobin levels (MCHL) are normal in the two males and white blood cells (WBC) are decreased in one male. In the female of the lot, WBC, MCHL, MCHC and LC are elevated while platelets (Pt) are decreased compared to their respective norm in contrast to the other parameters which are normal.

- An elevation of both Ht in the two males of lot 3 although there is a variation in WBC and Hg action, and a reduction in LC, RBC and Pt in one male out of two in contrast to MHCV, MCHL and MCHC which are normal in both males. In the female of the same batch, there is an increase in both MCHL, MCHC, Pt, and LC, and a decline in RBC, Ht, and MHCV, and normal WBC and Hg.

- In the males of lot 4, simultaneous elevation of RBCs, MHCV and LCs in both males, increase of Hg, Ht, MCHL and MCHC in one male, decrease of WBCs and normal value of Pt in both males. As for the female of the same batch, there is an elevated WBC, MCHL, MCHC, Pt and LCs, a reduced RBCs and Ht, and normal values of Hg and MHCV.

- In lot 5, there is a simultaneous elevated Hg, MCHL and MCHC in both males, and also Ht and MHCV in one male, a simultaneous decreased LC in both males and Pt in one male, and normal WBC and RBC in both males. For the female of the lot, MCHL and MCHC and LC are raised; whereas Ht, MHCV, RBC and Pt are reduced, and WBC and Hg are normal.

- An evolution of the MHCV in all individuals of lot 6, only of the MCHL and the Pt in the female, of the LC in both the male and the female and only of the Ht in a male. A reduction in WBC and Hg in both males, RBC in all individuals, and LC in one male from the same batch. MCHC in all individuals, MCHL and Pt in both males, Ht in one male and one female, and WBC and Hg in the female were all normal.

- An increase in Hg in both males, WBC, MHCV, MCHC and LC in one male and the female, Ht in one male, MCHL and Pt in the female of lot 7. The decrease in batch 7 was

observed in WBC in one male, RBC in one male and one female, Pt and LC in one male. RBC in one male, Hg in one female, Ht in one male and one female, MHCV in one male, MCHL in both males, MCHC in one male and Pt in one male were normal.

- In batch 8, there is an elevation of MHCV in both males, RBC and Hg in one male, MCHC and LC in one male and the female, and MCHL and Pt in the female. In the same batch, Pt was lowered in both males, WBC in one male, RBC and Ht in one male and one female, and MHCV in one female. WBC in one male and one female, Hg in one female, MCHL in both males, MCHC and LC in one male met their respective standards.

Table No. 1: Blood counts of control and untreated envenomed rabbits

	Lot 11: Male Controls						**Lot 12: Female Controls**						**Lot 1: Envenomed**		
	M7	M10	M16	AVE	Sd	**Reference values**	F1	F10	F12	AVE	Sd	**Reference values**	M18	M19	F4
WEIGHT (Kg)	1.9	2.4	1.5	1.91667	0.475	**1.442-2.392**	1.7	2	2	1.9	0.173	**1.73-2.07**	1.9	1.9	1.9
WBC	11	11	11	10.7667	0.462	**10.30-11.23**	7	5.2	5.4	5.86667	0.987	**4.88-6.85**	6.1	4.2	6.8
RBC	5.7	6	6,7	6.11667	0.499	**5.62-6.62**	6.1	5.9	5.9	5.98333	0.119	**5.86-6.10**	5.5	5.3	4.6
Hg	11	12	12	11.7333	0.416	**11.32-12.15**	11	11	5.9	9.37667	2.987	**6.39-12.36**	10	9	11
Ht	35	37	40	36.9667	2.335	**34.63-39.30**	37	36	38	37.0667	1.002	**36.06-38.07**	35	32	36.6
MHCV	62	61	59	60.5667	1.069	**59.50-61.64**	62	64	64	63.3333	1.422	**61.91-64.76**	**65**	**64**	**67**
MCHL	20	19	18	19.2	0.854	**18.35-20.05**	19	19	19	18.9667	0.351	**18.62-19.32**	20	19	**21**
MCHC	33	32	31	31.7	0.9	**30.8-32.6**	30	29	30	29.7333	0.635	**29.10-30.37**	31	31	30
Pt	769	590	400	586.33	184.5	**401.83-770.83**	264	270	277	270.333	6.506	**263.83-276.84**	694	441	**331**
LC	45	50	58	51.1	6.514	**44.59-57.61**	36	35	36	35.8	0.608	**35.19-36.41**	45	49	**53**
												State	**D**	**D**	**D**

RBC *= Red blood cells (x* $10^6/mm^3$*) ;* ***WBC*** *= White blood cells (x* $10^3/mm^3$*) ;* ***Hg*** *= Hémoglobin (g/dL) ;* ***Ht*** *= Hématocrit (%) ;* **MHCV** *= Mean hemoglobin cell volume(*μ^3*) ;* ***MCHL*** *= Mean corpuscular hemoglobin level (pg/L) ;* ***MCHC*** *= Mean corpuscular (or globular) hemoglobin concentration (%) ;* ***Pt*** *= Platelets (x* $10^3/mm^3$*) ;* **LC**av *= Lymphocytes*

*in absolute value ((%) ;**M**= Male ; **F**= Female ; **Ave** = average ; **Sd** = Standard deviation; Light grey = Low value ; Dark grey and bold = High value ; D= Death.*

Example: M10= Male number10; F1=Female number 1

Table No. 2: Blood counts of rabbits scarified as a preventive measure before envenomation

	Batch 2			**Batch 3**			**Batch 4**			**Batch 5**			**Batch 6**			**Batch 7**			**Batch 8**		
	M3	M13	F3	M14	M9	F11	M8	M11	F7	M1	M6	F6	M5	M12	F14	M15	M2	F13	M7	M4	F2
WEIGHT (Kg)	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
MHCV	59	**63**	62	61	60	60	**63**	**65**	62	60	**62**	60	**64**	**63**	**66**	**62**	61	**66**	**63**	**62**	60
MCHL	19.3	20	**20**	19	19	**20**	**21**	20	**20**	**21**	**21**	**20**	20	19	**20**	20	19	**21**	20	20	**20**
MCHC	**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 (x $10^6/mm^3$) ; **WBC** = White blood cells (x $10^3/mm^3$) ; **Hg** = Hémoglobin (g/dL) ; **Ht** = Hématocrit (%) ; **MHCV** = Mean hemoglobin cell volume(μ^3) ; **MCHL** = Mean corpuscular hemoglobin level (pg/L) ; **MCHC** = Mean corpuscular (or globular) hemoglobin concentration (%) ; **Pt** = Platelets (x $10^3/mm^3$) ; **LC**av = Lymphocytes in absolute value ((%) ; **M** = Male ; **F** = Female ; Light grey = Low value ; Dark grey and bold = High value.*

Example: M3 = Male number 3; F3 =Female number 3

Résult

The haemograms of the rabbits treated as a preventive measure and then envenomated in Table 3 allowed us to note, in comparison with lot 1 in Table 1 (untreated envenomated lot), that:

- in males, with the exception of the two males in lot 6, and to some extent one male in lots 4 and 8 which obey the observations made on the males of the untreated envenomed lot in Table 1, the various parameters of the various experimental lots in males are either generally normal or increased.

- in the females, apart from the female of lot 6 and to some extent successively the female of lot 3 and 7, and 2, 4 and 8 which respected the same findings as that of the untreated envenomed lot of Table 1, that of lot 5 is fundamentally different.

When the blood counts of the preventively treated and then envenomed rabbits in Table 3 are compared to those in Table 2 (rabbits scarified preventively prior to envenomation), it is noted that:

- in batch 2, in both males, RBC and Ht which had varied (increased) in Table 2 stabilized in Table 3, whereas WBC, MHCV and LC were the same for one out of two males, and MCHC and Pt for both males. Alone Hg does not obey any of these observations in the males. In the female of the batch, WBC and LC are higher while MCHC, MCHL and Pt have changed significantly in both parameter tables. Other than these observations, the rest parameters remained the same. All animals in this batch died one day after.

- in batch 3, WBCs, RBCs, Hg, Ht, MCHL, Pt and LCs were the same, whilst MHCV and MCHC varied substantially for one male in both tables. In the female of the lot, WBC, GR, Ht, MCHL, MCHC, and LC remained the same while Ht, MHCV, and Pt varied notably in both arrays. One male and one female from this batch survived throughout the experiment.

- in lot 4, parameters such as WBC, MHCV and MCHL are identical for both males in both tables, while Ht, Pt and LC are equal for one male in both tables. As for RBC, Hg, and MCHC, they changed dramatically for both males meanwhile Pt did for one male in both arrays. However, RBC, Hg, MCHC, Pt, and LC were the same for the female in the batch whilst WBC, Ht, MHCV, and MCHL varied considerably in both arrays. All animals survived for all four days of the experiment.

- in lot 5, RBC and Pt remained the same in males while Hg, Ht, MHCV and LC were the same for one male in both Tables. For WBC, MCHL and MCHC, they were particularly changed for males in both arrays. For the female in the lot, Hg, MCHC and LC are identical whereas WBC, RBC, Ht, MHCV, MCHL and Pt have largely varied in both arrays. All animals were found dead from the first day of the experiment.

- in batch 6, hematological parameters such as WBC, RBC, Hg, MHCV, MCHL, MCHC and Pt were the same for both males in Table 2 and 3 while Ht was the same for one male. LCs, on the contrary, were terribly altered. For the female, all parameters are similar in both tables. All animals died the next day of the experiment.

- in batch 7, in both males, hematological parameters like MHCV and MCHC are similar for both males in Table 2 and 3 in contrast to WBC, RBC, Hg, Ht, MCHL, Pt and LC which vary from one male to another and Hg which has evolved remarkably; while in the female, apart from hematological parameters like WBC and Ht which are different in the two Tables, the other parameters are similar. Two males survived the whole experiment.

- in lot 8, MHCV was the similar for both males while hematological parameters as WBC, Ht, MCHL, MCHC, Pt and LC were the same for one male of both tables. Out of these, RBCs and Ht varied significantly. At the female level, WBC, Hg, Ht, MCHL, MCHC, Pt and LC are equal in both arrays in contrast to RBC and MHCV which have changed diametrically. One male continued to exist throughout the experiment.

Table 3:

	Lot 2			**Lot 3**			**Lot 4**			**Lot 5**			**Lot 6**			**Lot 7**			**Lot 8**		
	M3	M13	F3	M14	M9	F11	M8	M11	F7	M1	M6	F6	M5	M12	F14	M15	M2	F13	M7	M4	F2
WEIGHT (Kg)	2.4	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	**12**	8.3	**6.9**	11	6.6	6	5.1	8.5	4.7	5.6	9.6	4.7	6.3	5.2	6.8	8.6	9.5	4.2	10	5.9	5
RBC	5.8	5.8	5.8	**7**	4.0	5.5	5.8	5.4	5.8	6.1	6.3	**6.3**	5.5	5.3	5.6	5.7	5.9	5.2	6.4	4.2	5.5
Hg	11	12	11	**13**	12	11	12	11	12	12	**13**	12	11	10	11	12	12	11	**14**	8.5	10.9
Ht	35	37	37	**45**	2.6	36	37	35	38	36	39	**40**	35	33	36.6	36	36	34	**43**	28	34.3
MHCV	60	**64**	64	**65**	**72**	**66**	**64**	**66**	**65**	59	**63**	63	**64**	**63**	**66**	**63**	61	**67**	**67**	**68**	62.3
MCHL	19	**21**	19	19	**33**	**20**	**21**	20	20	19	20	19	20	19	**20**	**21**	20	**21**	**21**	20	**19.7**
MCHC	**33**	32	30	**29**	**47**	**31**	32	30	**31**	32	32	**31**	31	31	30	**34**	32	**31**	31	30	**31.7**
Pt	606	603	**625**	**882**	99	47	247	561	**465**	128	429	498	694	441	**321**	338	185	**294**	149	9.8	**399**
LC	37	52	**41**	21	42	**45**	**58**	27	**40**	47	34	**40**	45	49	**52**	39	33	**44**	23	**71**	**40.5**
State	**D**	**D**	**D**	**D**	**S**	**S**	**S**	**S**	**S**	**D**	**D**	**D**	**D**	**D**	**D**	**S**	**S**	**D**	**S**	**D**	**D**

RBC *= Red blood cells (x* $10^6/mm^3$*) ;* ***WBC*** *= White blood cells (x* $10^3/mm^3$*) ;* ***Hg*** *= Hémoglobin (g/dL) ;* ***Ht*** *= Hématocrit (%) ;* **MHCV** *= Mean hemoglobin cell volume(*μ^3*) ;* ***MCHL*** *= Mean corpuscular hemoglobin level (pg/L) ;* ***MCHC*** *= Mean corpuscular (or globular) hemoglobin concentration (%) ;* ***Pt*** *= Platelets (x* $10^3/mm^3$*) ;* **LC**av = *Lymphocytes*

in absolute value ((%) ; ***M*** *= Male ;* ***F*** *= Female ; Light grey = Low value ; Dark grey and bold = High value ; D = Death; S = Survivor.*

Example: M7 = Male number 7; F2 =Female number 2

Results of blood counts of envenomated and curatively treated rabbits

The haemogram of the envenomed and then curatively treated rabbits in Table 4 compared with Lot 1 in Table 1 (untreated envenomed female) showed that:

- in lot 9, the reduction of RBCs was observed in all animals. It was followed by a decline in WBC in one female of the batch and its normalization in two females. In the same lot, the MHCV, MCHL and Pt maintained their upward trend for two females and standardization for one female. The Ht is found to be normal in two females and abnormal in one female, unlike the MCHC. Meanwhile, Hg remained normal. Finally, LCs decreased for all individuals in the batch in contrast to those in batch 1. One female persisted throughout the experiment.

- in batch 10, there is an increase in WBC for all animals in opposition to batch 1. RBC became stable for two females and stayed low for one female. MCHL and Pt were elevated in all animals as in batch 1 in contrast to MCHC and LC. Ht is considered normal for two females and not normal for one. As for MHCV, it was maintained high for one female as opposed to two. Ht was kept the same for all individuals. Two females survived all your experimental tests.

Table 4:

	Lot 1: envenomed	**Lot 9**			**Lot 10**		
	F4	F9	F8	F5	F15	F16	F17
WEIGHT (Kg)	1.9	1.7	2	2	1.9	1.74	2.2
WBC	6.8	6.5	2.9	5.3	**8.7**	**9.47**	**9**
RBC	4.6	5.5	5.3	5.63	4.78	5.95	6
Hg	11	11	11	11.37	9.8	12	11.3
Ht	36.6	35	37	36.87	30.7	37.9	36.6
MHCV	**67**	63	**70**	**65.47**	64.4	**64.8**	64.2
MCHL	**21**	19	**20.6**	**19.86**	**20.5**	**21**	**21.7**
MCHC	30	**31**	30	**30.73**	**319**	**31.5**	**30.9**
Pt	**331**	**454**	200	**327.3**	**721**	**441**	**440**
LC	**53**	2.9	1.4	3	6	6.7	6.35
State	**D**	D	D	S	S	D	S

RBC *= Red blood cells (x* $10^6/mm^3$*) ;* ***WBC*** *= White blood cells (x* $10^3/mm^3$*) ;* ***Hg*** *= Hémoglobin (g/dL) ;* ***Ht*** *= Hématocrit (%) ;* **MHCV** *= Mean hemoglobin cell volume(*μ^3*) ;* ***MCHL*** *= Mean corpuscular hemoglobin level (pg/L) ;* ***MCHC*** *= Mean corpuscular (or globular) hemoglobin concentration (%) ;* ***Pt*** *= Platelets (x* $10^3/mm^3$*) ;* **LC**av *= Lymphocytes in absolute value* ((%) *;* ***M*** *= Male ;* ***F*** *= Female ; Light grey = Low value ; Dark grey and bold = High value ; D = Death;*

S = Survivor.

Example: F15 =Female number 15

DISCUSSION

Result of the hemolytic power of the venom *in vitro*

The results of the *in vitro* hemolysis test showed that in general the venom of *Naja nigricollis* has a hemolytic effect on rabbit whole blood. However, these effects are dependent on its concentration. Indeed, hemolysis is more important when the concentration is high. This explains why the density is low with the 1.6 mg/mL concentration; whereas it is higher with the lowest concentration used (0.4 mg/mL). This gradual action of venom would imply that this effect would be a function of the quantity injected. Thus, venom has a dose-dependent action on red blood cells *in vitro*. These results are in agreement with those of [8]. This author indicates that the effect of Elapidae venom is proportional to the quantity of toxin molecules introduced into the organism.

Results of blood counts of control rabbits, untreated envenomed rabbits and preventively scarified rabbits

Results of the blood count of control rabbits

The results of this study reveal that with the exception of the WBC and Pt reference values which are higher in males than in females, the other reference values are roughly in the same ranges. In addition, the set of reference values of hematological parameters obtained in this study generally corroborates with those of [24], apart from the values of MHCVs of males which are below and MCHLs which are above those of the same author.

Blood count results of untreated envenomed rabbits

If the haemogram of envenomed and untreated rabbits of batch 1 reveals a general decrease of blood cells (WBC and RBC) in both males and females, this would mean that the venom of *Naja nigricollis* has a cytotoxic action on these blood cells. In fact, the work of [18, 21] proved the cytotoxic action of this venom. [12], also evoked the destructive action of snake venom on red blood cells which would be at the origin of anemia. According to [24], vitamin D deficiency could lead to changes in blood cells. The same author states that the more severe the vitamin D deficiency, the lower the hemoglobin level (anemia) and the higher the number of platelets and iron metabolism. This assertion would certainly explain the lower hemoglobin in both males and an elevation of platelets in the female. The author also stipulates that a high platelet count can lead to cardiovascular problems. Similarly, a lack of vitamin D can also affect white blood cells (cells of the immune system that protect against external aggression). The latter could be the cause of the decrease in white blood cells in the two males of batch 1.So, the action of the venom causes a blood disorder because it reduces in most cases the red blood cells in the two envenomed males and the female, depresses the hemoglobin of the two males and the hematocrit of one male, and augments the MHCVs of the two envenomed males and the female. According to [17], this disorder may be due to the presence of a large amount of active toxins on the cell membranes. Cytotoxins have many properties that have led to them being called variously: cardiotoxin, direct lytic factor, membrane toxin, hemolysin, cytolysin and cytotoxin (the name that eventually stuck). The main property of cytotoxins is to cause lysis of cell membranes. All these observations could justify the death of all the animals of this batch.

Results of the haemogram of rabbits scarified as a preventive measure

The results of the haemograms of the rabbits treated as a preventive measure before their envenomation showed, in an overall manner, an elevation of red blood cells (RBC), haemoglobin (Hg) and haematocrit (Ht) in the two male rabbits of batches 2 and 4, and one rabbit of batch 8. These different animals were treated with P1 (consisting of ash from both plants and viper skull), P3 (containing only the ash from *Millettia pinnata*) and P7 (including the ash from viper skull and *Millettia pinnata*), respectively. According to [23] and [3], unlike anemia, polyglobulia corresponds to an increase in total globular mass, which will be suspected when there is a proportional change in hemoglobin and hematocrit figures. In addition, the presence of *Millettia pinnata* in the preparation of the P1, P3 and P7 potions,

which caused an augmentation of the RBC and MHCV values in one rabbit from lot 2 and 8, and two rabbits from lot 4, would have a stimulating activity on hematopoiesis.[14], suggests that hematopoiesis is physiologically limited in adulthood to the medullary cavity of the bones of the axial skeleton and the epiphysis of the long bones, but in case of increased demand for cells, hematopoiesis described as extramedullary may extend to the liver, spleen and lymph nodes.

Table 2 also shows a simultaneous elevation of Hg and MCHC values in one rabbit batch 2, 4, 7 and 8, and two rabbits from batch 5. These animals were treated with P1 (ash of both plants and viper skull), P3 (ash of *Millettia pinnata*), P6 (ash of viper skull and *Mucuna pruriens*), P7 (ash of viper skull and *Millettia pinnata*), and P 4 (ash of viper skull), respectively. According to [23], the synthesis of Hg is carried out in the erythroblast and a certain high value of MCHC, this synthesis can be stopped. Therefore, it would be possible to say that P1, P3, P4, P6, and P7 can have a stimulatory 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 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 in them of the two organic products (*Millettia pinnata* and viper skull ash). Nevertheless, P4 (viper skull ash) would have more stimulatory action for Hg synthesis. In the same table, it is observed an augmentation of LC in one rabbit of the batch 2, 3 and 5, two rabbits of the batch 7 and 8 and three rabbits of the batch 4 with a simultaneous elevation of WBC in each female of the batches 2, 4 and 7. All of the above batches were treated with P1 (ash of both plants and viper skull), P2 (ash of *Mucuna pruriens*), P4 (ash of viper skull), P6 (ash of viper skull and *Mucuna pruriens*) and P7 (ash of viper skull and *Millettia pinnata*), respectively. According to [23], this elevation in WBCs indicates hyperleukocytosis; whereas an increase in LCs reveals hyperlymphocytosis. Hyperlymphocytosis is involved in cellular and humoral immunity i.e. antibody production. Thus, some animals treated with P1, P2, P3, P4, P6 and P7 obeyed hyperlymphocytosis, whilst each female of batches 2, 4 and 7 obeyed hyperleukocytosis and this would be due to the solicitation of P1, P3 and P6 respectively for their care. At the Pt level, an increase is observed for the female of batch 3, 4, 6, 7 and 8 treated with P2 (*Mucuna pruriens* ash), P3 (*Millettia pinnata* ash), P5 (*Mucuna pruriens* and *Millettia pinnata* ash), P6 (viper skull and *Mucuna pruriens* ash) and P7 (viper skull and *Millettia pinnata* ash), respectively. These different observations would suggest thrombocytosis (or hyperplaquettosis) according to [23].

Consequently, the action of the different ashes (P2, P3, P5, P6 and P7) applied to these various batches mentioned above is thrombocytosis. The same author states that the decrease in Pt in the female of batch 2 cared for with P1, in the male of batch 3 treated with P2, in the female and male of batch 5 groomed with P4, in the male of batch 7 cared for with P6 and in the two males of batch 8 treated with P7 would show a thrombocytosis Hence, P1, P2, P4, P6 and P7 used would have this thrombocytopenia in the different animals mentioned above. All these findings could explain the survival of all animals in the various experimental batches.

Results of the blood count of rabbits treated as a preventive measure and envenomed

Since only in two males of lot 6 cured for with P5 (ash of *Mucuna pruriens* and *Millettia pinnata*), one male and one female of lot 4 treated with P3 (ash of *Millettia pinnata*), a male from lot 3 groomed with P2 (*Mucuna pruriens* ash), a female from lot 7 treated with P6 (viper skull ash and *Mucuna pruriens*) and a male from lot 8 cured for with P7 (viper skull ash and *Millettia pinnata*), there was a general decrease in both blood cells (WBC and RBC), which would mean that the potions used had a significant effect on the cytolytic action of the *Naja nigricollis* venom, because out of a sample of twenty-one rabbits, only seven rabbits complied with these observations. Moreover, out of the seven dead rabbits, three remained alive despite the simultaneous decrease of their WBC and RBC. These were the male from lot 3, and the male and female from lot 4.

Concerning the other potions used, they have variable actions on the experimental batches. For example, the preventive action of P1 on batch 2 increased WBC in one male and one female, and stabilized RBC and Ht in the three animals, Hg in one male and one female, and Pt in the two males. In batch 3, the preventive action of P2 elevated MHCV and MCHC in all three animals and MCHL in one male and one female and normalized Hg in one male and one female. For batch 4, P3 increased the MHCV of all three animals and the LC of one male and one female, stabilized the Ht of all three animals, and the Hg and MCHL of one male and one female. These different findings reveal variability in hematological parameters showing clinical and blood disorders [12, 15]. Despite these clinical and blood disorders, some animals survived all the experimental tests. These were a female from lot 3, a male from lot 4, two males from lot 7 and a male from lot 8 treated with P2 (*Mucuna pruriens* ash), P3 (*Millettia pinnata* ash), P6 (viper skull and *Mucuna pruriens* ash) and P7 (viper skull and *Millettia pinnata* ash) respectively.

Results of the blood count of envenomed and curatively treated rabbits

The curative action of P1 (ashes of the two plants and the viper skull) on the blood cells (WBC and RBC) of the rabbits of lot 9 is not noticeable because their decrease is noted in a general way as in the case of the envenomed lot not treated. This observation shows that this potion could not efficiently protect these blood cells against the anemic action of the *Naja nigricollis* venom and would therefore justify the death of two females of the batch. However, one female was able to survive certainly because of the simultaneous increase in parameters such as MHCV, MCHL, MCHC and Pt. The solicitation of P1 also decreased the LCs of the three animals unlike in the female of the untreated envenomed lot.

As for the effect of P3 (*Millettia pinnata* ash) on lot 10, it is characterized by an increase in WBC and MCHC in all three animals, normalization of RBCs and MHCV in two animals and a decrease in LCs in all three animals. These numerous variations and standardization of hematological parameters would surely explain the survival of two out of three females.

CONCLUSION

Finally, the venom of *Naja nigricollis* at a certain dose has a hemolytic power whether *in vitro* or *in vivo*. This hemolytic power is dose-dependent. The cytotoxins of this venom are responsible for the damage caused on blood cells. This damage on the hematological parameters would cause anemia and would be at the origin of deaths of all the animals of the concerned batch. Nevertheless, the use of certain potions to prevent the action of the venom on the hematological parameters proves to be more or less effective. Thus, the use of:

- as a preventive measure before the envenomation of certain potions revealed that P3 (ash of *Millettia pinnata*) has a stimulating action on the hematopoiesis and on the synthesis of the hemoglobin, induces hyperlymphocytosis in the whole of the individuals, and a hyperleucocytosis and a thrombocytosis in the female of the batch; while P2 (*Mucuna pruriens* ash) has a hyperlymphocytosis effect and thrombocytosis in some individuals. All animals used survived.

- as a preventive measure and then envenomation afterwards showed a variability of action of the potions solicited indicating clinical and blood disorders. However, some animals remained alive despite these clinical and blood disorders.

- The curative treatment after envenomation allowed to notice that P3 (ash of *Millettia pinnata*) provokes the variation and the normalization of numerous hematological parameters with the survival of more animals used.

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CONFLICTS OF INTEREST

Authors declare that there are no conflicts of interest in relation to the publication on this manuscript.

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