Original Research Article

Screening of *Mucunapruriens*(L.) DC. and *Pongamiapinnata* (L.) Pierre(Fabaceae) minerals on biochemical parameters of *Oryctolaguscuniculus* asantivenom

ABSTRACT

Aims: The objective of this study was to evaluate the antivenom action of minerals from two plants on the biochemical parameters of rabbits.

Methodology: For its realization, thirty-six (36) rabbits including nineteen (19) males and seventeen (17) females were divided into three portions (E1, E2 and E3).In each portion, there are different batches with three rabbits per batch. So, for E1, each rabbit of the control lot and those of the experimental lot were collected separately in the red tubes (dry tubes).However, those from the experimental batch were injected 20-30 minutes after the injection of *Najanigricollis* venom. For E2, each rabbit in a given experimental batch was first scarified with a specific potion initially prepared and then separately harvested the following day.Then, each animal was separately envenomed and then separately sampled. In E3, the same procedure of E2 was observed with the only difference that this time, each animal of a given experimental lot was first separately envenomed and then scarified with a precise potion previously prepared and subsequently collected three days later.All the blood samples taken in the various red tubes were transported in a cooler for the determination of biochemical parameters.

Results: The venom has a dose-dependent action on the red blood cells *in vitro*, whereas the *in vivo* tests highlighted in a general way, the stabilizing and preventive action of the minerals from the potion P2 (*Mucunapruriens*), P3 (*Pongamiapinnata*), P4, P5 and P6, and the curative action of P1 and P3 (*Pongamiapinnata*) on some biochemical parameters. Indeed, their action was manifested by the survival of some envenomed animals with a greater activity for P3.

Conclusion: For the development of an antivenom, it would be advisable to use more *Pongamiapinnata*because of its effectiveness both preventively and curatively.

Keywords: Antivenomous, minerals, *Mucunapruriens*,*Pongamiapinnata*, biochemical parameters.

1. INTRODUCTION

“The annual number of ophidian bites exceeds 5 million and the number of people who die following an ophidian bite is 125,000” [13, 37]. “Worldwide, 500,000 to 5,000,000 people are estimated to be victims of snakebite envenomation, of which 50,000 to 150,000 die and 400,000 suffer serious functional sequelae: amputation, renal failure, neurological sequelae”[48, 37, 12].

Ophidian envenomations in Africa represent a public health problem, due to their severity, but also because of the difficulties of their management [37]. “In fact, in urban Africa, envenomed patients can be treated with serotherapy (immunotherapy). It comes from the specific study of venoms from rigorous biochemical and toxicological analysis” [10]. “However, immunotherapy, which remains the only specific treatment for ophidian envenomation, is very expensive, available only in a few pharmacies and is constantly in short supply” [19]. “For rural populations, venomous snake bites are a medical, social and economic problem because of their low income and the distance from medical centers [25]. In the Maghreb as well as in sub-Saharan countries, the venomous snakes considered more dangerous are represented by the Viperidae and Elapidae” [41, 33].

“Najamelanoleuca, *Najanigricollis*, *Najahaje...* are part of the Elapidae found in Africa [40]. The *Najanigricollis* has a venom containing toxins that can quickly immobilize a prey (neurotoxic)” [38]. “These toxins are polypeptides and proteins, of small size (PM < 30 kDa) and represent 50 to 70 % of the dry weight of Elapidae venoms” [38]. “These proteins can intervene alone or in synergy with other components of the venom to cause serious disruptions of biological systems of the body. These disturbances are at the origin of the edema, hematological, neurological, renal, pulmonary disorders and tissue necrosis characteristic of ophidian envenomation” [43, 6, 27]. These major facts push African populations to adopt traditional medicine as a therapeutic alternative.

“Numerous researches keep showing that medicinal plants contain various biologically active chemical principles that exert different pharmacological activities: antioxidant, anti-inflammatory, analgesic activities, etc”[7, 35, 16, 46]. “Among these bioactive substances, some of them may have effects on undoubtedly biochemical parameters” [42].

“According to the World Health Organization (WHO) 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 whose effectiveness is proven. As a matter of fact, it has been shown that the continent is full of nearly 5,000 medicinal species” [2, 45, 46].

“In West Africa, particularly in Benin, 80 % of people bitten by snakes claim to use traditional treatment rather than modern Western medicine” [9, 25]. “In Côte d'Ivoire, studies conducted by some

researchers have reported noteworthy results regarding the ethnomedical approach to treating snakebites with medicinal plants. According to these authors, the root of *Securidacalongepedunculata*Fresen. (Polygalaceae) can be used in the case of envenomations by Elapidae"[34, 51]. In addition, in the Bouaké region (Côte d'Ivoire), some populations use two Fabaceae, namely *Mucunapruriens* and *Pongamiapinnata*to treat cases of ophidian envenomations.

It is in the concern to exploit rationally this patrimony, 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. It aimed to know the antivenom effect *in vivo* of *Pongamiapinnata*and *Mucunapruriens* on some biochemical parameters of rabbits.

2. MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Biological materials

The plant material consisted of *Mucunapruriens* and *Pongamiapinnata* (Fabaceae). They were harvested in the month of December 2019 in Bouaké (Central Côte d'Ivoire).

2.1.2 Animal material

For this study, thirty-six rabbits, including nineteen (19) males and seventeen (17) females of the 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 in the animal house of the Laboratory of Animal Physiology of the University Jean LorougnonGuédé (UJLoG) of Daloa (Côte d'Ivoire), the weights of the rabbits varied between 1.45 and 2.4 Kg.Besides this animal model, viper skulls and *Najanigricollis* venom were also used. The viper skulls were provided by a medicodrugist while the *Najanigricollis* venom was supplied by the Pasteur Institute of Adiopodoumé (Côte d'Ivoire).

2.2 METHODS

2.2.1 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. Thereafter, the plant parts/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 collected were weighed with a precision balance. They were smooth except for the viper skull which was rough. The colors varied from gray to brown.

The combination of the ashes from the various biological products resulted in the following potions:
- P1 was composed of the ashes of the two plants and the skull of viper;
- P2, P3 and P4 were constituted respectively and only of ash of *Mucunapruriens*, *Pongamiapinnata*and skull of viper;
- P5 was formed by the ashes of *Mucunapruriens* and *Pongamiapinnata*;
- P6 was consisted of the ashes of skull of viper and *Mucunapruriens*;
- P7 was comprised of the ashes of viper skull and *Pongamiapinnata*.

2.2.2 Calculation of incineration efficiency

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

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

Ac :Ash content

2.2.3 Method of scarification of experimental batches

To scarify the experimental batches, the following potions:
- P1 was used for batch 4;
- P2 was utilized for lot 5;
- P3 was solicited for lots 6;
- P4 has been used for batch 7;
- P5 was served for lot 8;
- P6 was required for lot 9;
- P7 has been used for lot 10.

There were two males and one female in each experimental batch. 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. After that, 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.

2.2.4 Blood collection method

In general, blood samples were taken at the Agrovalorisation Laboratory of the Jean LorougnonGuédé University from the short saphenous vein and/or the femoral vein. The restraint method was performed by three individuals. 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 red tubes (dry tubes). The tubes obtained were kept in a cooler containing ice and then transported to the laboratory for analysis.

2.2.5 *In vitro* hemolysis test method

To carry out this test, two control batches (batch 1 and batch 2) were formed. Batch 1 consisted of three males and batch 2 of three females. Among these control batches, a blood sample was taken from one of them in order to perform the *in vitro* hemolysis test of *Najanigricollis* venom.To perform this test, ten (10) tubes were used including one (1) control tube and nine (9) experimental tubes. The stock solution was prepared in tube 1 by dissolving 1.6 mg of venom crystals in 1 mL of physiological water. In the remaining nine (9) tubes (tubes 2 to tubes 10), a volume of 0.5 mL of physiological water was added.The concentration ranges of venom 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 then discarded afterwards. Thus, the concentrations varied in the tubes from 1.6 mg/mL to $6.26.10^{-3}$ mg/mL. To these nine (9) experimental tubes and to the control tube (tube 10), 5 drops of rabbit whole blood collected and then manually homogenized were added. After mixing, the whole preparation was incubated at room temperature for 30 to 40 minutes for microscopic observation.This observation was performed at magnification 40 (X40). To do this, the preparations of tube 1 (SM); 2; 3 and tube 10 (control tube) were diluted separately to 1/5th. Then 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.

2.2.6 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. Indeed, according to [22], the median lethal dose for a 2 kg rabbit is 2 mg/Kg of body weight in intra-muscular injection.

3. RESULTS

3.1 Results 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 the venom tested *in vitro* on red blood cells. These figures showed that in general, the density of red blood cells varied compared to the control depending on the concentrations tested. Thus, it was higher when the concentration was low (0.4 mg/mL) and low when the concentration was high (1.6 mg/mL).

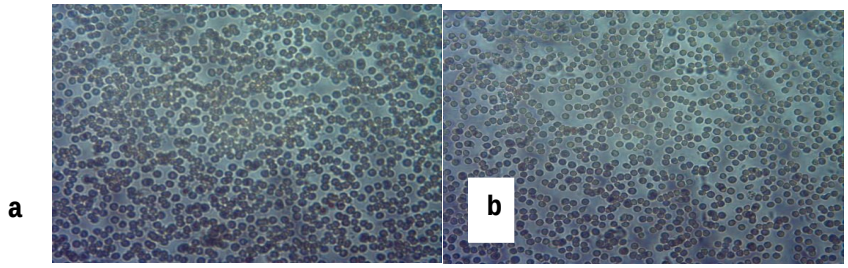

Fig. 1. Hemolysing power of venom on red blood cells (at magnification 40)
1.a: control red blood cells; 1.b: red blood cells at 0.4 mg/mL venom concentration

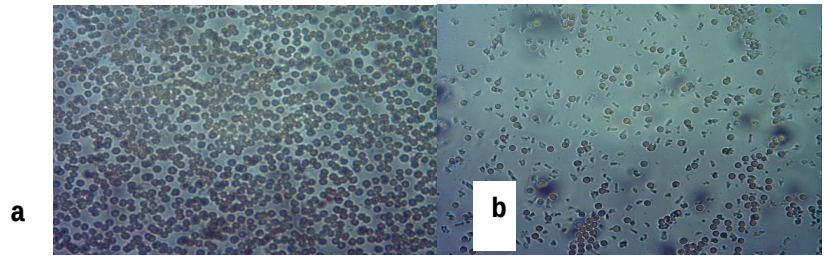

Fig. 2.Hemolysing power of venom on red blood cells (at magnification 40)
2.a: control red blood cells; 2.b: red blood cells at 0.8 mg/mL venom concentration

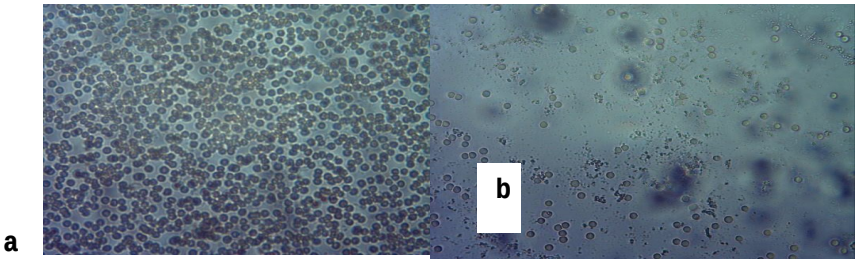

Fig. 3.Hemolysing power of venom on red blood cells (at magnification 40)
3.a: control red blood cells; 3.b: red blood cells at a concentration of 1.6 mg/mL venom

3.2 Results of biochemical parameters of control rabbits

The results of the biochemical parameters of the control rabbit batches were recorded in table 1 below. In this table, the reference value of biochemical parameters of:

- blood glucose was 193.8 ± 24.77 mg/dL for the male and 152.32 ± 17.35 mg/dL for the female;
- creatine levels were 18.38 ± 1.44 mg/L for males and 15.87 ± 1.83 mg/L for females;
- uremia were 45.93 ± 3.55 mg/L for males and 39.66 ± 4.57 mg/L for females;
- GOT value was 87.1 ± 75.99 IU/L for males and 124.57 ± 46.92 IU/L for females;
- GPT value was 81.12 ± 44.09 mg/L for the male and 13.76 ± 15.81 mg/L for the female;
- triglycerides were 344.71 ± 127.18 mg/dL for the male and 154.16 ± 37.64 mg/dL for the female;

- total cholesterol were 94.12 ± 23.08 mg/dL for males and 112.96 ± 16.26 mg/dL for females;
- HDL cholesterol for males was 26.16 ± 33.86 mg/dL and for females 68.07 ± 12.69 mg/dL;
- LDL cholesterol readings were 122.67 ± 184.78 mg/dL for males and 20.87 ± 12.88 mg/dL for females;
- sodium content was 132.87 ± 7.72 mmol/L for males and 141.9 ± 1.75 mmol/L for females;
- chlorine were 107.43 ± 10.22 mmol/L for males and 101.5 ± 0.62 mmol/L for females,
- potassium levels were 3.93 ± 0.13 mmol/L for males and 4 ± 0 mmol/L for females.

Table 1.Biochemical parameters of control batches

Lots	Individuals	Gly	Creat.	Urea	Transaminases		TG	CT	HDLc	LDLc	Ionogram		
					GOT	GPT					Na	Cl	K
Biochemical parameters of the male control batch													
Lot 1	MW 07	185.2	17.38	43.45	121.41	126.52	198.02	69.33	9.09	20.63	124.5	116.4	3.78
	MW 16	221.72	17.74	44.35	139.88	78.37	411.98	98.04	4.23	11.42	134.4	96.3	4
	MW 10	174.47	20.03	50	0	38.47	424.12	114.98	65.15	335.97	139.7	109.6	4
	Average	193.8	18.38	45.93	87.1	81.12	344.71	94.12	26.16	122.67	132.87	107.43	3.93
	Standard deviation	24.77	1.44	3.55	75.99	44.09	127.18	23.08	33.86	184.78	7.72	10.22	0.13
Reference value		169.03-218.57	16.94-19.82	42.38-49.48	11.11-163.09	37.03-125.21	217.53-471.89	71.04-117.2	-7.7-60.02	-62.11-307.45	125.15-140.59	97.21-117.65	3.8-4.06
Biochemical parameters of the female control batch													
Lot 2	FW 01	134.02	14	35	80.78	0.56	120.62	106.19	64.83	17.24	143.6	100.8	4
	FW 10	154.4	15.94	39.85	118.83	31.29	194.87	131.47	57.32	35.18	142	101.7	4
	FW 12	168.53	17.66	44.14	174.09	9.44	147	101.21	82.07	10.2	140.1	102	4
	Average	152.32	15.87	39.66	124.57	13.76	154.16	112.96	68.07	20.87	141.9	101.5	4
	Standard deviation	17.35	1.83	4.57	46.92	15.81	37.64	16.26	12.69	12.88	1.75	0.62	0
Reference value		134.97-169.67	14.04-17.7	35.09-44.23	77.65-171.49	-2.05-29.57	116.52-191.8	96.7-129.22	55.38-80.76	7.99-33.75	140.15-143.65	100.88-102.12	4

Source : Okou et al.(2020)

Gly : Glycemia(mg/dL) ; Creat. : Creatinine (mg/L) ; Urea (mg/L) ; Transaminases (GOT and GPT in UI/L) ; TG : Triglycerides(mg/dL) ; TC : Total Cholesterol(mg/dL) ; HDLc : HDL cholesterol (mg/dL) ; LDLccholesterol (mg/dL) ; Na : Sodium (mmol/L) ; Cl : Chloride(mmol/L) ; K : Potassium (mmol/L). MW:Male Witness;FW:Female Witness

3.3 Results of the biochemical parameters of the envenomed rabbits

In table 2 are the results of the biochemical parameters of the envenomed rabbit lot. Analysis of these results revealed that:

- inthe M1 male, creatinine, uremia, triglycerides and potassium (K) were above their respective reference values, whereas sodium was below its reference value. Apart from these parameters the others were within their respective norms.
- inmale M2, blood glucose and triglycerides were respectively superior to their norms, meanwhile transaminases (GOT and GPT), sodium (Na) and potassium (K) were inferior to their respective reference values. Independently of these, the other parameters were normal.
- atthe F1 level, blood glucose, creatinine, urea, transaminases, LDLc and ionogram (Na, Cl and K) were elevated compared to their respective norms, in contrast to HDLc. As for triglycerides and total cholesterol, their values were normal.

Table 2. Biochemical parameters of the envenomed batch

Lots		Gly	Creat.	Urea	Transaminases		TG	TC	HDLc	LDLc	Ionogramm			State
					GOT	GPT					Na	Cl	K	
MW	Ave	193.8	18.38	45.93	87.1	81.12	344.71	94.12	26.16	122.67	132.87	107.43	3.93	
	Sd	24.77	1.44	3,55	75.99	44.09	127.18	23.08	33.86	184.78	7.72	10.22	0.13	
Reference value		169.03-218.57	16.94-19.82	42.38-49.48	11.11-163.09	37.03-125.21	217.53-471.89	71.04-117.2	-7.7-60.02	-62.11-307.45	125.15-140.59	97.21-117.65	3.8-4.06	
FW	Ave	152.32	15.87	39.66	124.57	13.76	154.16	112.96	68.07	20.87	141.9	101.5	4	
	Sd	17.35	1.83	4.57	46.92	15.81	37.64	16.26	12.69	12.88	1.75	0.62	0	
Reference value		134.97-169.67	14.04-17.7	35.09-44.23	77.65-171.49	-2.05-29.57	116.52-191.8	96.7-129.22	55.38-80.76	7.99-33.75	140.15-143.65	100.88-102.12	4	
Lot 3	M1	181.22	11.82	29.63	58.02	48.83	120.55	90.81	9.08	58.01	171.1	110.02	3.1	Death
	M2	154.15	19.39	48.51	219.22	172.52	77.73	104.27	20.09	67.73	165.4	106.42	7.54	Death
	F1	172.2	20.64	51.6	381.85	171.08	174.01	116	29.96	49.33	229.3	130.01	9.72	Death

Legend

Male

Lower value

Higher value

Female

Lower value

Higher value

Glyc : Glycemia(mg/dL) ; Creat : creatinine(mg/L) ; Urea (mg/L) ; Transaminases (GOT and GPT en UI/L) ; TG : Triglycerides(mg/dL) ; TC : Total Cholestérol (mg/dL) ; HDLc : HDL cholesterol (mg/dL) ; LDLc : LDL cholesterol (mg/dL) ; Na : Sodium (mmol/L) ; Cl : Chlorine(mmol/L) ; K : Potassium (mmol/L). MW: Male witness;FW: Female witness; Ave:Average;Sd: Standard deviation

Example: M1= Male number 1; F1= Female number 1

3.4 Results of mineral tests on biochemical parameters of rabbits treated as a preventive measure before envenomation

The results of the biochemical parameters of the preventively treated rabbits were reported in table 3. Analysis of the biochemical parameters in this table in comparison with the reference values in table 1 revealed that:

- at the level of glycemia, it was normal in a male from lot 4 to lot 10 and in the female from lot 5, lot 9 and lot 10; while it was increased in a male from lot 4 and the female from lot 4, 6, 7 and 8 contrary to a male from lot 5 to 10.
- at the level of creatinine, it was normal in the two males of lot 4 and 7, one male of lot 5 and 8, the female of lot 5, 7 and 9; whereas it was elevated in the female of lot 4, 6 and 8 in contrast to a male of lot 5, 8 and a female of lot 10, and two males of lot 6, 9 and 10.
- with regard to urea, the respect of its norm was observed in two males of lot 4 and 7, one male of lot 5 and 8, and in the female of lot 7, 9 and 10; meanwhile, it was high in the female of lot 4, 6 and 8 in opposition to that of a male of lot 5 and 8, of two males of lot 6, 9 and 10, and of a female of lot 5
- at the GOT level, it was normal in the three individuals of lot 4, two males of lot 6, 7 and 9, one male of lot 8 and 10; at the time it was augmented in two males of lot 5, in one male of lot 8 and 10, and in the female of lot 5, 6, 7, 8, 9 and 10.
- in GPT, it was greater in the female of lots 4, 5, 6, 7, 8, 9 and 10, and in one male of lots 5 and 8. Apart from these observations, it was normal in the individuals of the other batches.
- triglycerides were lower in one male from lots 4, 5, 6, 9 and 10, and in two males from lots 7 and 8, and vice versa in the female from lots 4, 5, 6, 7 and 9. Except for these findings, they met their standard in the other cases.
- total cholesterol was high in the females of lots 4, 6, 7 and 9, and in one male of lots 5 and 10, although the opposite was true in one male of lots 6 and 7. Excluding these remarks, it was normal in the animals of the various constituted batches.
- the HDLc level was decreased in the female of lots 4, 5, 6, 7, 8, 9 and 10 in contrast to a male of lot 5. In addition to these observations, the respect of its norm was noticed in the animals of the different batches made.
- LDLc was elevated in one male from batch 4 and in the female from batches 4, 5, 6, 7, 8, 9 and 10, but was normal in the other individuals from the various batches.
- in terms of sodium, it was increased in the female of batches 7 to 10, and two males of batches 7 to 10; however, it was low in a female of batches 4 to 6, and a male of batch 5. Except for these remarks, it was normal in the other animals.
- at the level of chlorine, it was reduced in the female of lot 7; whilst it was increased in the female of lots 4, 5, 6, 8, 9 and 10, and a male of lot 7. Besides these remarks, it obeyed its norm in the other individuals of the different batches realized.
- in terms of potassium, it was normal in the two males of lot 4, and one male of lots 5, 9 and 10. It was lower in the female of lots 4 to 7, one male of lots 5, 7 and 8, and the two males of lot 6, in contrast to one male of lots 7 to 10, and in the female of lots 8 to 10.

Table 3. Biochemical parameters of rabbits treated with minerals as a preventive measure

Lots	Individuals	Gly	Creat.	Urea	Transaminases		TG	TC	HDLc	LDLc	Ionogram		
					GOT	GPT					Na	Cl	K
Lot 4 (P1)	M.3	180.09	17.6	44	40.59	66.17	177.34	104.59	42.25	26.88	137.6	112.8	3.8
	M.13	222.91	17.66	44.15	99.37	99.86	312.9	96.05	33.93	259.76	132.9	115.3	3.84
	F.3	192.27	19.14	47.85	133.67	181.21	335.66	137.4	20.85	49.42	132.9	109.1	3.95
Lot 5 (P2)	M.14	125.11	18.57	46.42	370.57	106.26	350.59	245.9	141.86	33.93	123.7	107.4	3.81
	M.9	187.08	7.1	17.75	253.07	212.66	77.96	76.72	40.15	21.03	132.6	112.1	3.66
	F.11	157.81	15.26	34.66	201.12	179.57	202.69	122.34	15.32	62.9	135.9	115.6	3.81
Lot 6 (P3)	M.8	179.3	16.24	40.6	103.78	112.65	349.48	102.06	11.9	317.17	127.8	114.7	3.78
	M.11	119.6	14.19	35.47	111.43	50.37	147.56	62.86	13.05	121.94	125.7	117.1	3.41
	F.7	190.88	23.84	59.6	231.24	192.35	281.81	138.31	23.96	230.19	129.4	117.6	3.65
Lot 7 (P4)	M.1	131.61	17.01	42.52	69.39	84.95	146.13	64.88	7.23	28.22	183.3	127.5	6.93
	M.6	206.25	19.03	47.57	71.55	66.34	116.55	116.09	13.06	79.72	183.6	110	2.91
	F.6	188.94	15.8	39.5	175.82	109.94	319.96	163.16	14.94	84.23	165.3	98.7	3.36
Lot 8 (P5)	M.5	181.2	11.82	29.53	57.97	48.83	120.15	90.81	8.88	57.9	171.1	110.6	3.1
	M.12	154.25	19.39	48.47	219.32	172.52	75.73	104.27	25.89	67.23	165.4	105.2	7.54
	F.14	172.19	20.64	51.6	381.85	171.08	173.81	116	30.91	50.33	229.3	129.6	9.83
Lot 9 (P6)	M.15	141.39	14.53	36.32	71.68	76.64	234.56	98.29	13.39	37.99	188.3	111.5	4.03
	M.2	186.8	16.83	42.07	129.51	122.88	211.36	93.53	13.33	37.93	171.8	112.9	6.27
	F.13	142.83	14.83	37.05	604.59	245.89	287.09	155.16	24.08	76.66	181.9	118.1	5.66
Lot 10 (P7)	M.7	170.88	16.68	41.7	104.16	79.86	158.49	90.82	13.15	79.86	160.37	113.33	4.2
	M.4	132.7	16.7	41.75	170.54	89.28	243.76	136.36	54.16	33.38	165.1	115.47	4.92
	F.2	158.78	16.34	40.85	342.09	484.52	133.92	118.42	16.73	74.91	168.4	114.1	5.84
Reference value	M	169.03-218.57	16.94-19.82	42.38-49.48	11.11-163.09	37.03-125.21	217.53-471.89	71.04-117.2	-7.7-60.02	-62.11-307.45	125.15-140.59	97.21-117.65	3.8-4.06
	F	134.97-169.67	14.04-17.7	35.09-44.23	77.65-171.49	-2.05-29.57	116.52-191.8	96.7-129.22	55.38-80.76	7.99-33.75	140.15-143.65	100.88-102.12	4

Gly : Glycemia (mg/dL) ; Creat. : Creatinine (mg/L) ; Urea (mg/L) ; Transaminases (GOT and GPT in UI/L) ; TG : Triglycerides (mg/dL) ; TC : Total Cholesterol (mg/dL) ; HDLc : HDL cholesterol (mg/dL) ; LDLccholesterol (mg/dL) ; Na : Sodium (mmol/L) ; Cl : Chloride (mmol/L) ; K : Potassium (mmol/L).M:Male;F: Female
Example: M7= Male number 7; F2= Female number 2

3.5 Results of mineral tests on biochemical parameters of rabbits treated as a preventive measure and then envenomed

The biochemical parameters of the rabbits treated as a preventive measure and then envenomated in table 4 made it possible to note in comparison with lot 3 in table 2 (untreated envenomated lot) that :

- in general, the batches are characterized by the death of all animals in batches 4, 7 and 8, and the survival of one male in batch 10, one male and one female in batch 5, two males in batch 9, and all individuals in batch 6.
- therewas considerable variation in blood glucose, creatinine, urea and total cholesterol in all animals of the different batches made. However, exceptions were observed for transaminases, triglycerides, HDLc, LDLc and ionograms in the different batches. Thus at the level of:
- GOT, apart from the animals of lot 4 that obeyed the results of table 2, the GOT values obtained in the other lots differed from those of table 2;
- GPT, besides the animals of lot 4 and in a certain sense those of lot 9 and 10, the GPT values got in the other cases were different from those of table 2;
- triglycerides, except for the animals of lot 8 which respected the observations of table 2, the females of lots 6, 7, 9 and 10 allowed to have variable triglyceride values contrary to the different males of these lots. As for the remaining batches, their triglyceride values were variable;
- HDLc, excluding lots 7, 8 and 10 and to some extent lot 5 which followed the remarks in table 2, the HDLc values achieved in the other cases varied;
- LDLc, the results were exactly the same as in table 2, with one exception, a male from lot 4;
- ionogram, with the certain exception of the animals of lot 4 and those of lot 10 which respected the results of table 2, the animals of the other lots allowed to have discordant results.

When the biochemical parameters of the preventively treated and then envenomed rabbits in table 4 were compared to those in table 3 (rabbits scarified preventively prior to envenomation), it was noted that in the:

- lot 4; outside of triglycerides and chlorine, which were the same in both tables, the other parameters (blood glucose, creatinine, urea, GOT, GPT, total cholesterol, HDLc, LDLc, sodium and potassium) varied either in one animal or in two or even in three for this lot;
- in lot 5; except for GOT and LDLc that were identical in both tables, the other parameters differed for the same lot from one animal of the same sex to another;
- from lot 6; all biochemical parameters studied for a given animal of a constituted lot changed in both tables;
- from lot 7; with the exception of triglycerides, HDLc and sodium which were the same in both tables, the other biochemical parameters studied varied in one or two or three animals for that lot from one table to another;

- of lot 8; other than the triglycerides, HDLc, LDLc and sodium which did not change between the two tables, the other biochemical parameters studied were modified in one or two or three animals for this lot from one table to another;
- from lot 9; apart from LDLc and sodium which were the same in both tables, the other biochemical parameters studied were not in one or two or three animals for this lot from one table to another;
- of lot 10; independently of HDLc, LDLc and ionogram (sodium, chlorine and potassium) which were the same in both tables, the other biochemical parameters studied were not identical in one animal or two or three for this lot from one table to another.

Table 4. Biochemical parameters of rabbits treated as a preventive measure and then envenomed

Lots	Individuals	Gly	Creat.	Urea	Transaminases		TG	TC	HDLc	LDLc	Ionogram			State
					GOT	GPT					Na	Cl	K	
Lot 4 (P1)	M.3	339.32	33.87	13.55	83.69	75.12	151.98	54.08	61.23	166.55	156.5	107.8	8.22	Death
	M.13	350.66	44.6	17.84	203.54	143.31	273.09	55.14	132.6	360.67	144.9	97.4	1.27	Death
	F.3	308.17	48.75	19.5	320.61	327.61	428.6	99.51	98.25	267.24	197	118.4	8.57	Death
Lot 5 (P2)	M.14	39.47	18.7	46.75	675.38	0	237.11	209.47	35.51	96.59	154.8	100.5	4.31	Death
	M.9	147.35	17.04	42.6	295.44	271.03	112.04	28.85	9.05	24.62	163.1	104.2	3.8	Survivor
	F.11	82.38	16.95	42.37	225.4	0	86.36	99.06	80.09	217.84	142.3	98.7	1.81	Survivor
Lot 6 (P3)	M.8	115.06	13.76	34.4	426.76	0	105.8	61.85	71.92	195.62	164.3	99.3	4.72	Survivor
	M.11	324.68	39.15	15.66	90.16	106.74	96.66	44.86	60.79	165.35	147.5	102	5.69	Survivor
	F.7	78.19	17.18	42.95	121.08	563.75	116.16	116.53	23.94	65.12	153.4	101.2	5.16	Survivor
Lot 7 (P4)	M.1	85.53	16.57	41.42	146.05	251.56	59.11	62.03	51.64	140.46	145.2	99.1	2.26	Death
	M.6	341.19	38.52	15.41	175.89	134.53	140.2	47.55	59.56	162	146.1	101.8	4.86	Death
	F.6	82.1	17.86	44.65	0	0	264.21	100.19	15.04	40.91	150.3	98.9	4.71	Death
Lot 8 (P5)	M.5	93.48	16.49	41.22	709.54	0	45.24	58.87	48.63	132.27	154.9	105.2	2.16	Death
	M.12	148.47	17.76	44.4	227.57	321.61	84.41	53.91	12.01	32.67	150.3	107.9	4.1	Death
	F.14	117.64	12.61	31.52	369.78	0	174.93	47.24	15.78	42.92	149.7	101.2	2.7	Death
Lot 9 (P6)	M.15	106.07	16.97	42.42	175.2	0	78.81	76.29	64.96	176.69	153.2	102.1	4.53	Survivor
	M.2	85.13	23.25	58.12	0	632.38	60.41	80.66	64.42	175.22	142.4	100.6	3.38	Survivor
	F.13	350.66	44.6	17.84	203.54	143.31	273.09	55.14	132.6	360.67	144.9	97.4	1.27	Death
Lot 10 (P7)	M.7	51.29	53.43	133.87	0.2	445.12	124.06	24.94	23.41	63.67	147.3	102.6	3.91	Survivor
	M.4	84.36	19.12	47.8	245.5	219.36	55.19	46.84	34.81	94.68	153.6	102.8	4.08	Death
	F.2	103.42	13.75	34.37	84.84	524.72	84.76	40.25	16.56	45.04	148.3	103.8	4.41	Death

Gly : Glycemia (mg/dL) ; Creat. : Creatinine (mg/L) ; Urea (mg/L) ; Transaminases (GOT and GPT in UI/L) ; TG : Triglycerides (mg/dL) ; TC : Total Cholesterol (mg/dL) ; HDLc : HDL cholesterol (mg/dL) ; LDLccholesterol (mg/dL) ; Na : Sodium (mmol/L) ; Cl : Chloride (mmol/L) ; K : Potassium (mmol/L). M:Male;F: Female
Example: M1= Male number 1; F14= Female number 14

3.6 Results of mineral tests on biochemical parameters of rabbits treated curatively after envenomation

The biochemical parameters of the envenomed and then curatively treated rabbits in table 5 in comparison with lot 3 in table 2 (untreated envenomed lot) showed that:

- in lot 11, the increase of GPT and ionogram (sodium, chlorine and potassium) was noticed in two females of the lot as well as those of lot 3 (envenomated not treated). Similarly, it was found that the two individuals in table 5 did not survive like the one in table 2. The elevation of GPT and ionogram were followed by a regression of total cholesterol in both individuals. Apart from these common parameters, the others varied. As for the surviving female, these biochemical parameters such as blood glucose, creatinine, transaminases (GPT and GOT), LDLc and sodium were rising like those of batch 3 (untreated envenomated). Except for these parameters, the others changed.
- in batch 12, elevated creatinine, urea, GPT, LDLc, sodium and chlorine in one female were followed by a decrease in HDLc. These observed parameters were identical to those of batch 3 (untreated envenomed). It was also observed that one of the two females in table 5 did not survive like the one in table 2. In contrast to this finding, the second female was found alive. Regardless of these parameters, the others changed. In the second female of the batch that survived, there was an increase in GPT, sodium and potassium as well as those of batch 3 (envenomed untreated). In addition to these parameters, the others varied.

Table 5. Biochemical parameters of envenomed and curatively treated rabbits

Lots	Individuals	Gly.	Creat.	Urea	Transaminases		TG	CT	HDLc	LDLc	Ionograms			State
					GOT	GPT					Na	Cl	K	
Lot 11 (P1)	F.9	152.94	13.1	32.53	159.15	155.83	126.06	52.96	14.31	13.44	166.4	104.8	8.11	Death
	F.5	322.64	24.8	9.92	278.13	196.18	229.63	93.59	101.53	53.87	152.4	96.9	2.38	Survivor
	F.8	87.1	19.15	47.87	150.02	130.5	103.7	85.29	76.11	11.56	153.5	106.6	4.49	Death
	F.15	97.34	8.83	22.87	100.95	440.48	215.59	95.43	15.99	36.32	185.2	122.7	6.65	Survivor
Lot 12 (P3)	F.16	58.19	20.1	49.8	100	73	96.91	77.89	23.8	34.71	154.9	106.1	3.4	Death
	F.17	99.93	22.4	55.7	168	209	233.88	137.97	59.8	11.4	186.4	101.2	4.1	Survivor
Lot 3 envenomed	F1	172.2	20.64	51.6	381.85	171.08	174.01	116	29.96	49.33	229.3	130.01	9.72	Death

Gly : Glycemia (mg/dL) ; Creat. : Creatinine (mg/L) ; Urea (mg/L) ; Transaminases (GOT and GPT in UI/L) ; TG : Triglycerides (mg/dL) ; TC : Total Cholesterol (mg/dL) ; HDLc : HDL cholesterol (mg/dL) ; LDLccholesterol (mg/dL) ; Na : Sodium (mmol/L) ; Cl : Chloride (mmol/L) ; K : Potassium (mmol/L).F: Female
Example: F17= Female number 17

4. DISCUSSION

4.1 Results of the hemolytic power of the venom *in vitro*

"The results of the *in vitro* hemolysis test showed that in general the venom of *Najanigricollis* has a hemolytic effect on rabbit whole blood. However, these effects are dependent on its concentration. In fact, hemolysis is more important when the concentration is high. This explains why the density of red blood cells is low with the concentration of 1.6 mg/mL; 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" [11]. This author indicates that the effect of Elapidae venom is proportional to the quantity of toxin molecules introduced into the organism.

4.2 Results of biochemical parameters of control rabbits

Comparison of the result of the reference values in table 1 showed that with the exception of GOT, total cholesterol, HDLc and sodium, the biochemical parameters of males were generally higher than those of females. Besides these exceptions, the biochemical parameters obtained varied from male to female animals. This observation was confirmed by [18] who stated that "the variation in blood content of these different biochemical elements is a function of sex, reproductive cycle, age and growth of rabbits". "Nevertheless, all the biochemical parameters obtained from the control batches during this study were with few exceptions identical to those of" [14].

4.3 Results of the biochemical parameters of the envenomed rabbits

At M1 and F1, there is an elevation in both creatinine and urea values. According to [1],[29], [52] and [44], an increase in serum urea concentration associated with creatinine would indicate renal failure. Therefore, it can be inferred that the M1 and F1 males would obey this assertion. There was also an increment in triglycerides in M1 and M2. According to [3], [17] and [44], "this rising value of triglycerides would be consistent with hypertriglyceridemia and would be a risk factor for predicting cardiovascular disease.The reason is that triglycerides are the main constituents of cell membranes and the increase in their concentration would probably be the result of apoptosis". Accordingly, M1 and M2 would obey this principle. LDLc is also elevated in F1. This increase could promote the risk of coronary mortality because according to [39], [28] and [44], its reduction would induce the opposite effect. Regarding the elevation of transaminases in F1, it could indicate hepatocellular damage or bile flow disturbance according to [15] and [44].The high value of ionogram (Na, Cl and K) in F1 and blood glucose in F1 and M2 would mean a heart problem and diabetes respectively according to [50]. In M2, the decrease in transaminases would reveal a vitamin B6 deficiency according to [8]. Concerning dyskalemia (potassium variation) in M1 and M2, they would be the basis of neuromuscular and cardiac signs according to [30] or even hemodynamic according to [4].These authors believe that potassium

plays a major role in maintaining the resting membrane potential. These authors generally stipulate that dyskalemia (hyperkalemia or hypokalemia) is life-threatening. HDLc decay in F1 would designate according to [21], [15] and [44], “hypocholesterolemia. It can be observed in liver damage, severe infections, anemia, treatment with particular drugs (hormones such as clofibrate and androsterone), mental retardation and congenital acyl transferase deficiency. Thus, these different pathologies mentioned above would be related to the action of the venom on the vital organs of the various animals involved, which would lead to their death”.

4.4 Results of mineral tests on biochemical parameters of rabbits treated as a preventive measure before envenomation

“The increase in blood glucose in M13 and F3 of lot 4, F7 of lot 6, F6 of lot 7 and F14 of lot 8 treated respectively with P1 (ash of both plants and viper skull), P3 (ash of *Pongamiapinnata*), P4 (ash of viper skull) and P5 (ash of *Mucunapruriens* and *Pongamiapinnata*) would indicate, according to [50] and [44], hyperglycemia”. These authors suggest that hyperglycemia is due to insulin-dependent or non-insulin-dependent diabetes, pancreatic diseases: acute or chronic pancreatitis, endocrine diseases: pheochromocytoma, hypercorticism, corticosteroid therapy, and hypothyroidism. The decrease in M14 of lot 5, M11 of lot 6, M1 of lot 7, M12 of lot 8, M15 of lot 9 and M4 of lot 10 treated respectively with P2 (*Mucunapruriens* ash), P3 (*Pongamiapinnata*ash), P4 (viper skull ash), P5 (ash of *Mucunapruriens* and *Pongamiapinnata*), P6 (ash of viper skull and *Mucunapruriens*) and P7 (ash of viper skull and *Pongamiapinnata*) would reflect hypoglycemia according to the same authors. It would be due to malnutrition or prolonged fasting, secretion by the body of an excess of insulin: insulinoma, polyadenomatosis, endocrine insufficiency: adrenal, pituitary, and a hepatic disorder: acute hepatitis. Also, according to [31] and [44]; “blood glucose is also low in anorexia and high in diabetes. Hence, it can be said that anorexia would be observed in M14 of batch 5, M11 of batch 6, M1 of batch 7, M12 of batch 8, M15 of batch 9 and M4 of batch 10; whereas diabetes would be seen in M13 and F3 of batch 4, F7 of batch 6, F6 of batch 7 and F14 of batch 8”.

“The elevation of creatinine in F3 of batch 4, F7 of batch 6, F6 batch 7 and F14 of batch 8 treated with P1 (ash of both plants and viper skull), P3 (ash of *Pongamiapinnata*) respectively, P4 (ash of viper skull) and P5 (ash of *Mucunapruriens* and *Pongamiapinnata*) could be explained by the reduction of creatinuria i.e. the elimination of creatinine by the kidneys because this excretion by the kidneys is more specific to creatinine than urea” [52, 44]. “This decrease in creatinuria would thus show a dysfunction of the kidney. Regarding the decline in creatinine in M9 of batch 5, M8 and M11 of batch 6, M5 of batch 8, M15 and M2 of batch 9, and M7, M4 and F2 of batch 10 treated with P2 (*Mucunapruriens* ash), P3 (*Pongamiapinnata* ash) respectively, P5 (*Mucunapruriens* and *Pongamiapinnata*ash), P6 (viper skull and *Mucunapruriens* ash) and P7 (viper skull and *Pongamiapinnata*ash), it could be a sign of cachexia” [47, 49, 52, 44].

The higher urea in F3 of batch 4, F7 of batch 6 and F14 of batch 8 treated with P1 (ash of both plants and viper skull), P3 (ash of *Pongamiapinnata*) and P5 (ash of *Mucunapruriens* and *Pongamiapinnata*), respectively, would indirectly reveal renal and liver function dysfunction [26, 15, 44]. “Relatively to its low in M9 and F11 of lot 5, M8 and M11 of lot 6, M5 of lot 8, M15 and M2 of lot 9, M7 and M4 of lot 10 treated with P2 (*Mucunapruriens* ash), P3 (*Pongamiapinnata*ash) respectively, P5 (ash of *Mucunapruriens* and *Pongamiapinnata*), P6 (ash of viper skull and *Mucunapruriens*) and P7 (ash of viper skull and *Pongamiapinnata*) would show hypo-uremia. This hypo-uremia would reflect severe liver failure or alternatively a deficiency in urea cycle enzyme” [15, 44].

According to [1, 29, 52, 44], the rise in serum urea concentration associated with that of creatinine would indicate renal failure. As such, it is possible to deduce that F3 of batch 4, F7 of batch 6 and F14 of batch 8 treated with P1 (ash of both plants and viper skull), P3 (ash of *Pongamiapinnata*) and P5 (ash of *Mucunapruriens* and *Pongamiapinnata*) respectively would obey this assertion.

“The enhancement of GOT in M14, M9 and F11 from lot 5, F7 from lot 6, F6 from lot 7, M12 and F14 from lot 8, F13 from lot 9 and M4 and F2 from lot 10 treated P2 (*Mucunapruriens* ash), P3 (*Pongamiapinnata* ash), P4 (viper skull ash), P5 (*Mucunapruriens* and *Pongamiapinnata* ash) respectively, P6 (ashes of viper skull and *Mucunapruriens*) and P7 (ashes of viper skull and *Pongamiapinnata*) would show the destruction of hepatocytes although in addition to the liver, this enzyme can be found in the heart, skeletal muscle, lungs and kidneys” [20, 23, 44].

“The increase in GPT in F3 of lot 4, M9 and F11 of lot 5, F7 of lot 6, F6 of lot 7, M12 and F14 of lot 8, F13 of lot 9 and F2 of lot 10 treated with P1 (ash of both plants and viper skull), P2 (ash of *Mucunapruriens*), P3 (ash of *Pongamiapinnata*), P4 (ash of viper skull), respectively, P5 (ash of *Mucunapruriens* and *Pongamiapinnata*), P6 (ash of viper skull and *Mucunapruriens*) and P7 (ash of viper skull and *Pongamiapinnata*) would reveal hepatic cell necrosis because GPT is a cytosolic enzyme secreted in liver cells. It is released into the bloodstream in case of hepatic cell necrosis” [32, 20, 23, 44].

According to [15] and [44], “elevated transaminases (GOT and GPT) could reflect hepatocellular damage or bile flow disturbance. Therefore, elevated transaminases (GOT and GPT) would be observed in acute hepatitis. Hence, M9 and F11 of batch 5, F7 of batch 6, F6 of batch 7, M12 and F14 of batch 8, F13 of batch 9 and F2 of batch 10 treated respectively with P2 (*Mucunapruriens* ash), P3 (*Pongamiapinnata*ash), P4 (viper skull ash), P5 (ash of *Mucunapruriens* and *Pongamiapinnata*), P6 (ash of viper skull and *Mucunapruriens*) and P7 (ash of viper skull and *Pongamiapinnata*) would obey this observation”.

Decrease in triglycerides was observed in M3 of batch 4, M9 of batch 5, M11 of batch 6, M1 and M6 of batch 7, M5 and M12 of batch 8, M2 of batch 9 and M7 of batch 10 treated with P1 (ash of both plants and viper skull) respectively, P2 (ash of *Mucunapruriens*), P3 (ash of *Pongamiapinnata*), P4 (ash of

viper skull), P5 (ash of *Mucunapruriens* and *Pongamiapinnata*), P6 (viper skull ash and *Mucunapruriens*) and P7 (viper skull ash and *Pongamiapinnata*) in opposition to their elevation in F3 of lot 4, F11 of lot 5, F7 of lot 6, F6 of lot 7 and F13 of lot 9 treated with P1 (ash of both plants and viper skull), P2 (ash of *Mucunapruriens*), P3 (ash of *Pongamiapinnata*), P4 (ash of viper skull) and P6 (ash of viper skull and *Mucunapruriens*) respectively. This increase in triglycerides is consistent with hypertriglyceridemia in females from the above batches. According to [3], [17] and [44], this hypertriglyceridemia would be a risk factor for predicting cardiovascular disease. In effect, triglycerides are the main constituents of cell membranes and the elevation of their concentration would likely be the result of apoptosis. So, F3 from batch 4, F11 from batch 5, F7 from batch 6, F6 from batch 7 and F13 from batch 9 would be exposed to cardiovascular diseases in contrast to M3 from batch 4, M9 from batch 5, M11 from batch 6, M1 and M6 from batch 7, M5 and M12 from batch 8, M2 from batch 9 and M7 from batch 10 treated respectively with P1 (ash from both plants and from viper skull), P2 (ash of *Mucunapruriens*), P3 (ash of *Pongamiapinnata*), P4 (ash of viper skull), P5 (ash of *Mucunapruriens* and *Pongamiapinnata*), P6 (ash of viper skull and *Mucunapruriens*) and P7 (ash of viper skull and *Pongamiapinnata*) respectively.

The increase in total cholesterol was noticed in F3 of batch 4, M14 of batch 5, F7 of batch 6, F6 of batch 7, F13 of batch 9 and M4 of batch 10 treated with P1 (ash of both plants and viper skull), P2 (ash of *Mucunapruriens*), P3 (ash of *Pongamiapinnata*) respectively, P4 (viper skull ash), P6 (viper skull and *Mucunapruriens* ash) and P7 (viper skull and *Millettiapinnata* ash), in contrary to their regression in M11 of lot 6 and M1 of lot 7 treated with P3 (*Pongamiapinnata*ash) and P4 (viper skull ash), respectively. The reduction designates according to [21], [15] and [44], “hypocholesterolemia. It can be observed in liver damage, severe infections, anemia, treatment with particular drugs (hormones such as clofibrate and androsterone), mental retardation and congenital acyl transferase deficiency. As for their elevation, according to the authors, it indicates hypercholesterolemia. It is observed in hepatic disorders, renal disorders, pancreatic disorders and thyroid disorders”. According to [28] and [44], “hypercholesterolemia is characterized by chronic inflammation of the arterial wall that develops in response to damage affecting the vascular endothelium. Atherosclerotic lesions develop in large and medium caliber arteries, which are approximately 3 to 0.5 cm in diameter”.

The lowering of HDLc found in F3 of batch 4, F11 of batch 5, F7 of batch 6, F6 of batch 7, F14 of batch 8, F13 of batch 9, and F2 of batch 10 treated with P1 (ash of both plants and viper skull), P2 (ash of *Mucunapruriens*), P3 (ash of *Pongamiapinnata*), respectively, P4 (viper skull ash), P5 (*Mucunapruriens* and *Pongamiapinnataash*), P6 (viper skull and *Mucunapruriens*ash), and P7 (viper skull and *Pongamiapinnata* ash) was in antagonism to M14 from lot 5 treated with P2 (*Mucunapruriens* ash). According to [36] and [44, “a 1 % increase in HDLc is associated with a 3 to 4 % decrease in coronary risk, and a level below 0.40 g/L in humans is considered a risk factor for cardiovascular disease”. Similarly, the work of [5] and [44] showed “that the increase in HDLc levels would indicate a protective factor for the heart muscle, thus revealing its beneficial effect against cardiovascular complications, particularly atherosclerosis. Therefore, M14 of lot 5 treated with P2 (*Mucunapruriens*

ash) would be more protected against atherosclerosis than F3 of lot 4, F11 of lot 5, F7 of lot 6, F6 of lot 7, F14 of lot 8, F13 of lot 9 and F2 of lot 10".

The elevation of LDLc was seen in M8 of lot 6 treated with P3 (*Pongamiapinnata* ash) as well as in F3 of lot 4, F11 of lot 5, F7 of lot 6, F6 of lot 7, F14 of lot 8, F13 of lot 9, and F2 of lot 10 treated with P1 (ash of both plants and viper skull), P2 (*Mucunapruriens* ash), P3 (*Pongamiapinnata* ash), P4 (viper skull ash), P5 (*Mucunapruriens* and *Pongamiapinnata* ash), P6 (viper skull and *Mucunapruriens* ash), and P7 (viper skull and *Pongamiapinnata* ash) respectively. [39], [28], and [44], suggested that a 0.38 g/L reduction in LDLc would be associated with a one-third decrease in coronary mortality. Hence, M8 in batch 6, F3 in batch 4, F11 in batch 5, F7 in batch 6, F6 in batch 7, F14 in batch 8, F13 in batch 9, and F2 in batch 10 would be more prone to coronary mortality because of their increased.

The increase in sodium observed in M1, M6, and F6 of lot 7, M5, M12, and F14 of lot 8, M15, M2, and F13 of lot 9, and M7, M4, and F2 of lot 10 treated with P4 (viper skull ash), P5 (*Mucunapruriens* and *Pongamiapinnata*ash), respectively, P6 (viper skull ash and *Mucunapruriens*) and P7 (viper skull ash and *Pongamiapinnata*) was in contradiction to F3 of lot 4, F11 and M14 of lot 5 and F7 of lot 6 treated with P1 (ash of both plants and viper skull), P2 (*Mucunapruriens* ash) and P3 (*Pongamiapinnata* ash) respectively. This elevation and regression of sodium would determine according to [50] "respectively a hypernatremia and a hyponatremia. According to the same author, hypernatremia can be the consequence of a decline in the quantity of water, diarrhea, vomiting, significant sweating, significant water loss (diabetes insipidus, diabetes mellitus), intense exercise. So, M1 and M6 and F6 of lot 7, M5, M12 and F14 of lot 8, M15, M2 and F13 of lot 9, and M7, M4 and F2 of lot 10 would conform to this assertion. As for hyponatremia, it can be the consequence of a decrease in the amount of salt during digestive losses (vomiting, diarrhea), renal losses, skin losses (extensive burning, sweating), an increase in the amount of water during inappropriate secretions of anti-diuretic hormones, excessive water intake, renal failure, cardiac failure, hepatic failure. Therefore, F3 of batch 4, F11 and M14 of batch 5 and F7 of batch 6 would comply with these remarks".

The lowering of chlorine was detected in F6 of lot 7 cared for by P4 (viper skull ash) in contrast to F3 of lot 4, F11 of lot 5, F7 of lot 6, M1 of lot 7, F14 of lot 8, F13 of lot 9 and F2 of lot 10 processed by P1 (ash of both plants and viper skull), P2 (ash of *Mucunapruriens*) respectively, P3 (*Pongamiapinnata* ash), P4 (viper skull ash), P5 (*Mucunapruriens* and *Pongamiapinnata*ash), P6 (viper skull and *Mucunapruriens* ash) and P7 (viper skull and *Pongamiapinnata* ash) would, according to [50], announce hypochloremia and hyperchloremia respectively. Hypochloremia can be the consequence of a depletion of salt due to digestive losses (vomiting, diarrhea), renal losses, skin losses (extensive burns, sweating), an increment of water during inappropriate secretions of antidiuretic hormones, excessive water intake, renal failure, cardiac failure, liver failure. Whereas hyperchloremia can be the consequence of decreased water intake, diarrhea, vomiting, heavy sweating, heavy water loss (diabetes insipidus, diabetes mellitus), intense exercise. Thus F6 of batch 7 would approve the case of hypochloremia while the others would approve hyperchloremia.

Potassium depreciation noticed in F3 of lot 4, M9 and F11 of lot 5, M8, M11 and F7 of lot 6, M6 and F6 of lot 7, and M5 of lot 8 tended by P1 (ash of both plants and viper skull) respectively, P2 (ash of *Mucunapruriens*), P3 (ash of *Pongamiapinnata*), P4 (ash of viper skull), and P5 (ash of *Mucunapruriens* and *Pongamiapinnata*) was the reverse in M1 from lot 7, M12 and F14 from lot 8, M2 and F13 from lot 9, M4 and F2 from lot 10 processed with P4 (viper skull ash), P5 (*Mucunapruriens* and *Pongamiapinnata*ash), P6 (viper skull and *Mucunapruriens* ash), and P7 (viper skull and *Pongamiapinnata*ash) respectively. These variations would report hypokalemia and hyperkalemia respectively according to [50]. The same author reveals that dyskalemia (abnormalities: hypokalemia or hyperkalemia) are frequently the cause of cardiovascular disorders. However, hypokalemia can be encountered during digestive losses (vomiting, diarrhea), in hyperaldosteronism (corticoid treatment), renal tubular acidosis, hyperglycemia, metabolic alkalosis. Meanwhile, hyperkalemia can be in exogenous potassium intake, adrenal insufficiency (or antialdosterone treatment), hemolysis, crush syndrome, chemotherapy, intense exercise, chronic renal failure. Therefore, F3 of batch 4, M9 and F11 of batch 5, M8, M11 and F7 of batch 6, M6 and F6 of batch 7, and M5 of batch 8 would show hypokalemia, while M1 of batch 7, M12 and F14 of batch 8, M2 and F13 of batch 9, M4 and F2 of batch 10 would show hyperkalemia.

4.5 Results of mineral tests on biochemical parameters of preventively treated and envenomed rabbits

The death of all the animals of lot 4; 7 and 8, and the survival of a male and a female of lot 5, of all the animals of lot 6, of two males of lot 9 and of a male of lot 10 treated respectively by P1, P4 and P5 (for those who died) and, P2, P3, P6 and P7 (for those who survived). These results would indicate that in the first case P1, P4 and P5 could not have had a preventive action on the various animals constituted against the action of the venom. This state of affairs was at the origin of the death of all these animals. In the second case, P2, P3, P6 and P7 were able to prevent the life of one, two or even three animals, depending on the batch considered against the action of the venom.

If the comparison of the biochemical parameters of the batches treated as a preventive measure and then envenomated with those of the untreated envenomated batch allowed considerable variability in their parameters, this would prove that the venom caused clinical and blood disorders. These clinical and blood disorders obtained during this study were confirmed by the work of [22] and[24]. Thus, according to the work of [43],[6], and [27], “the toxins from the venom can intervene alone or in synergy with other components of the venom to cause serious disturbances of the biological systems of the organism. These disturbances are the cause of edema, hematological, neurological, renal, pulmonary and tissue necrosis characteristic of ophidian envenomation”.

4.6 Results of mineral tests on biochemical parameters of rabbits treated curatively after envenomation

The mortality of two females from batch 11 treated with P1 (i.e., approximately 66.67 %) and one female from batch 12 treated with P3 (i.e., approximately 33.33 %) would mean that these various potions could not have had a complete curative action against the venom on these respective animals. However, the survival of one female from lot 11 cured by P1 (i.e., about 33.33 %) and two females treated by P3 (i.e., about 66.67 %) would show that these various potions could have had a partial curative effect against the venom on these animals. Consequently, it is possible to say that P3 is more curatively active than P1 on the animals used.

If the comparison of the biochemical parameters of the envenomed and then curatively treated batches to those of the untreated envenomed batch allowed variable biochemical parameters from one batch to another, this would prove that the venom induced clinical and blood disorders. These are corroborated by the work of [22] and[24]. Similarly, the work of [43],[6]and [27] have shown that "toxins from the venom can act alone or in synergy with other components of the venom to cause serious disruption of the biological systems of the body. These disturbances are the cause of edema, hematological, neurological, renal, pulmonary and tissue necrosis characteristic of ophidian envenomation".

5. CONCLUSION

The general objective of this study was to determine the antivenom effects of*Mucunapruriens* and *Pongamiapinnata*minerals on some biochemical parameters in rabbits. These two plants are traditionally considered to have antivenom therapeutic properties. Thus, this study corroborates the objective of our course which is to develop the medicinal plants of our heritage. The results of this study showed that the venom has a dose-dependent action on red blood cells *in vitro*. As for the *in vivo* tests, they demonstrated that in general, the minerals from the potion P2 (*Mucunapruriens*), P3 (*Pongamiapinnata*), P4, P5 and P6 stabilize certain biochemical parameters on a preventive basis. As a curative measure, P1 and P3 (*Pongamiapinnata*) more or less preserve certain parameters because some animals were able to survive in both cases of treatment against the action of the venom, with a greater activity for P3. Therefore, for the development of an anti-venomous phytomedicine, it would be more advisable to use *Pongamiapinnata*since it has proven to be effective both as a preventive and curative agent.

ETHICAL APPROVAL

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

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