

Evaluation of Some Biochemical Parameters of Envenomed *Oryctolagus cuniculus*

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Abstract

Original Research Article

The general objective of this study was to evaluate the action of *Naja nigricollis* venom on certain biochemical parameters of rabbits. To carry out this study, three batches of three rabbits were formed with two control batches and one experimental batch. Each control lot is composed of three rabbits (male or female) while the experimental lot is composed of two males and one female. Each rabbit from the control lots was collected separately from the red tube (dry tube) and transported to the laboratory for analysis. The rabbits from the experimental batch were also collected separately a few minutes after the injection of *Naja nigricollis* venom for analysis of biochemical parameters. However, prior to these experiments, an *in vitro* hemolysis test of *Naja nigricollis* venom was performed on whole blood to verify the hemolysing power of *Naja nigricollis* venom. The results of this study reveal that:- the venom of *Naja nigricollis* has a dose-dependent hemolysing power *in vitro* and *in vivo*;- with a few exceptions, the biochemical parameters of males are generally higher than those of females;- the venom of *Naja nigricollis* causes kidney failure and cardiovascular disease in some animals, promotes a risk of coronary death and diabetes in others, causes neuromuscular and cardiac signs due to dyskalemia in others, and finally, a hepatocellular damage or disturbance of bile flow, a heart problem and vitamin B6 deficiency in one type of animal. - the venom of *Naja nigricollis* is fatal.

Keywords: Venom, *Naja nigricollis*, biochemical parameters.

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INTRODUCTION

The annual number of ophid bites exceeds 5 millions and the number of people who die from an ophid bite is 125,000 [9, 22]. Worldwide, 500,000 to 5,000,000 people are estimated to be victims of snakebite poisoning, of whom 50,000 to 150,000 die and 400,000 suffer serious functional sequelae: amputation, kidney failure, neurological sequelae [28, 22, 8].

Ophidic poisoning in Africa is a public health problem, not only because of its seriousness but also because of the difficulties in treating it [22]. Indeed, in urban Africa, patients with ophidic envenomas can be treated with serotherapy (immunotherapy). It comes from the specific study of venoms based on rigorous biochemical and toxicological analyses [6]. Nevertheless, immunotherapy, which remains the only specific therapy for ophid envenomation, is still very expensive, is available in only a few pharmacies and is

constantly in short supply [14]. For rural populations, venomous snake bites are a medical, social and economic problem because of their low income and the distance from medical centers [16]. In the Maghreb, as in sub-Saharan countries, venomous snakes considered more dangerous are represented by Viperidae and Elapidae [25, 21].

Naja melanoleuca, *Naja nigricollis*, *Naja haje*... are among the Elapidae found in Africa [26]. The *Naja nigricollis* has a venom containing toxins that can rapidly immobilize a (neurotoxic) prey [23]. These toxins are polypeptides and proteins, small in size (PM < 30 kDa) and represent 50 to 70% of the dry weight of Elapidae venoms [23]. These proteins can act alone or in synergy with other components of the venom to cause serious disturbances in the body's biological systems. These disturbances are responsible for the oedema, hematological, neurological, renal, and

pulmonary and tissue necrosis that are characteristic of ophid envenomation [27, 4, 17].

This study was conducted in order to understand the action that the venom of *Naja nigricollis* can have on certain biochemical parameters.

MATERIALS AND METHODS

MATERIAL

Animal material

For this study, nine rabbits including five males and four females of Hyplus breed, aged two and a half months were purchased from a breeder in the locality of Daloa (Côte d'Ivoire). After the acclimatization period the weight of the rabbits varied between 1.45 and 2.4 kg. Beside this animal model, the venom of *Naja nigricollis* (spitting cobra) was also used and supplied by the Pasteur Institute of Adiopodoumé (Côte d'Ivoire).

METHODS

In vitro hemolysis test method

Two control lots (lot 1 and lot 2) were established for this test. Lot 1 consists of three males and lot 2 consists of three females. Among these control lots, a blood sample was taken from one of them in order to perform the in vitro hemolysis test of *Naja nigricollis* venom. To perform this test, 10 tubes were used, including a control tube and 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 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 ratio 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 from tube 2 and homogenizing it. This procedure was repeated up to tube 9. From tube 9, a volume of 0.5 mL was collected and subsequently discarded. Thus, concentrations in the tubes ranged from 1.6 mg/mL to $6.26 \cdot 10^{-3}$ mg/mL. To these 9 experimental tubes and to the control tube (tube 10), 5 drops of rabbit whole blood

was added and homogenized manually. After homogenization, all preparations were incubated at room temperature for 30 to 40 minutes for microscopic observation. This observation was carried out at magnification 40 (X40). For this purpose, the preparations in tubes 1 (SS); 2; 3 and tube 10 (control tube) were diluted distinctly to 1/5th. Then each dilution was spread between slide and slide by putting the dilution of tube 10 (control) and an experimental dilution (e.g. tube 1). This last operation was also performed for tube 2 and tube 3.

Method of blood collection

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

Method of carrying out experimental tests

For the experimental tests, a quantity of 2 mg of venom crystals were dissolved in 0.5 mL of physiological solution to have a concentration of 4 mg/mL. This concentration was injected into the rabbits. Indeed, according to [15], the median lethal dose for a 2 kg rabbit is 2 mg/kg body weight by intramuscular injection.

RESULTS AND DISCUSSION

RESULTS

Result of the hemolysing power of 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 show that, in general, the density of red blood cells varies compared to the control depending on 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).

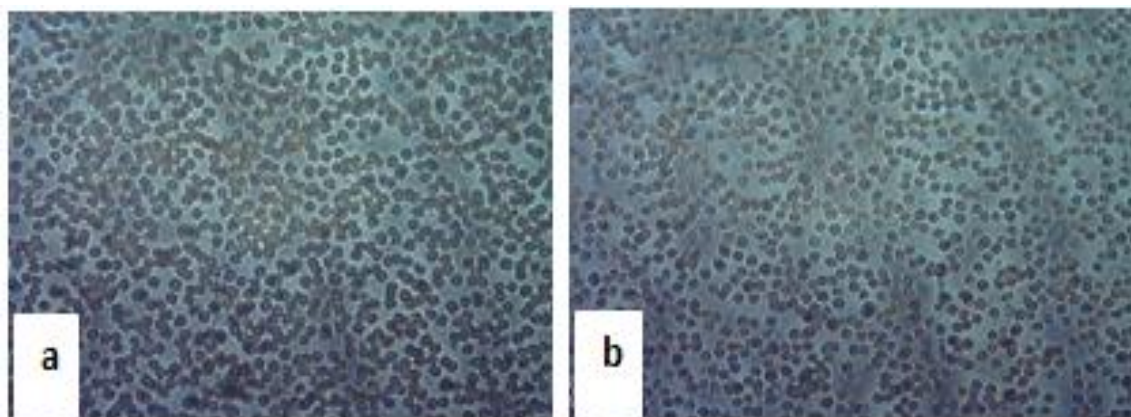


Fig-1: Hemolysing power of venom on red blood cells (at 40 magnifications) 1.a: control red blood cells; 1.b: red blood cells at a concentration of 0.4 mg/mL venom

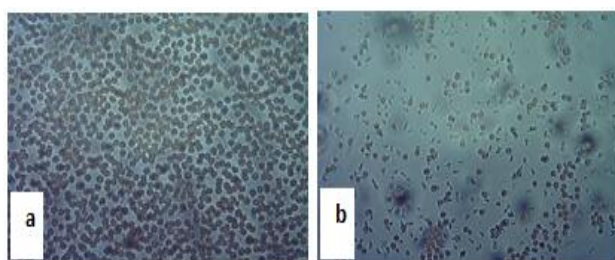


Fig-2: Hemolysing power of venom on red blood cells (at 40 magnifications) 2.a: control red blood cells; 2.b: red blood cells at a concentration of 0.8 mg/mL venom

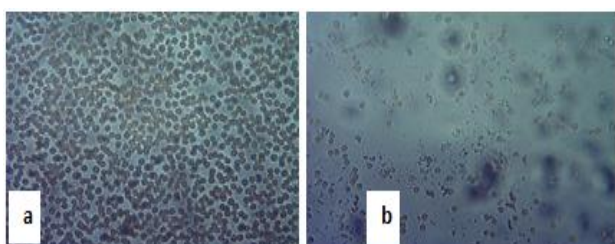


Fig-3: Hemolysing power of venom on red blood cells (at magnification 40) 3.a: control red blood cells; 3.b: red blood cells at a concentration of 1.6 mg/mL venom

Results of biochemical parameters of control rabbit batches

The results of the biochemical parameters of the control rabbit batches are reported in Table 1 below.

In this table, the reference value for the biochemical parameters of:

- blood glucose is 193.8 ± 24.77 mg/dL for males and 152.32 ± 17.35 mg/dL for females;
- creatine values are 18.38 ± 1.44 mg/L for males and 15.87 ± 1.83 mg/L for females;
- uremia levels are 45.93 ± 3.55 mg/L for males and 39.66 ± 4.57 mg/L for females;
- GOT/ASAT values are 87.1 ± 75.99 IU/L for males and 124.57 ± 46.92 IU/L for females;
- the GPT/ALAT value is 81.12 ± 44.09 mg/L for males and 13.76 ± 15.81 mg/L for females;
- triglycerides are 344.71 ± 127.18 mg/dL for males and 154.16 ± 37.64 mg/dL for females;
- total cholesterol is 94.12 ± 23.08 mg/dL for males and 112.96 ± 16.26 mg/dL for females;
- HDLc value is 26.16 ± 33.86 mg/dL for males and 68.07 ± 12.69 mg/dL for females;
- LDLc values are 122.67 ± 184.78 mg/dL for males and 20.87 ± 12.88 mg/dL for females;
- sodium is 132.87 ± 7.72 mmol/L for the male and 141.9 ± 175 mmol/L for the female;
- chlorine values are 107.43 ± 10.22 mmol/L for males and 101.5 ± 0.62 mmol/L for females,
- potassium is 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	TC	HDLc	LDLc	Ionogram		
					GOT	GPT					Na	Cl	K
Biochemical parameters of the male control batch													
Lot 1	MW 1	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 2	221.72	17.74	44.35	139.88	78.37	411.98	98.04	4.23	11.42	134.4	96.3	4
	MW 3	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 1	134.02	14	35	80.78	0.56	120.62	106.19	64.83	17.24	143.6	100.8	4
	FW 2	154.4	15.94	39.85	118.83	31.29	194.87	131.47	57.32	35.18	142	101.7	4
	FW 3	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

Glyc : glycemia (mg/dL) ; Creat : creatinine (mg/L) ; Urea (mg/L) ; Transaminases (GOT and GPT en UI/L) ; TG : triglycerides (mg/dL) ; TC : total cholesterol (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

Result of the biochemical parameters of the batch of envenomed rabbits

In table 2 are the results of the biochemical parameters of the batch of rabbits that were envenomed. Analysis of these results reveals that:

- all individuals in the experimental batch died;
- in male M1, creatinine, uremia, triglycerides, LDLc and potassium (K) are above their respective reference values, whereas sodium is below its reference value. Apart from these parameters the others are in their respective norm;

- at the M2 male level, blood glucose and triglycerides are beyond their respective standards, while transaminases (GOT and GPT), sodium (Na) and potassium (K) are within their respective reference values. Regardless of these, the other parameters are normal;
- at the F1 level, blood glucose, creatinine, urea, transaminases, LDLc and ionograms (Na, Cl and K) have high values compared to their respective standards, unlike HDLc. Triglycerides and total cholesterol are normal.

Table-2: Biochemical parameters of the poisoned batch

Lots	Gly	Creat.	Urea	Transaminases		TG	TC	HDLc	LDLc	Ionogram			
				GOT	GPT					Na	Cl	K	
Lot 1 (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 -	16.94 -	42.38-49.48	11.11-163.09	37.03-125.21	217.53 -	71.04-117.2	-7.7-60.02	-62.11-307.45	125.15-140.59	97.21-117.65	3.8-4.06
Lot 2 (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 -	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	M 1	181.22	11.82	29.63	58.02	48.83	120.55	90.81	9.08	58.01	171.1	110.02	3.1
	M 2	154.15	19.39	48.51	219.22	172.52	77.73	104.27	20.09	67.73	165.4	106.42	7.54
	F 1	172.2	20.64	51.6	381.85	171.08	174.01	116	29.96	49.33	229.3	130.01	9.72

Legend
Male
Lower value
Higher value
Female
Lower value
Higher value

This Legend applies to the other tables that follow.

Status
Death
Death
Death

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

DISCUSSION

Result of the hemolysing power of venom in vitro

The results of the in vitro hemolysis test showed that, in general, the venom of *Naja nigricollis* has a hemolysing effect on rabbit whole blood. But 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

concentration of 1.6 mg/mL, whereas it is higher with the lowest concentration used (0.4 mg/mL). This gradual venom action 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 [7]. This author indicates that the effect of Elapidae venom is proportional to the quantity of toxin molecules introduced into the organism.

Result of the biochemical parameters of the control rabbit batches and the envenomed rabbit batch

Comparison of the results of the reference values in Table 1 shows that with the exception of the parameters of GOT, total cholesterol, HDLc and sodium, the parameters of males are generally higher than those of females. According to [13], the variation in the blood content of these different biochemical elements is a function of sex, reproductive cycle, age and growth of rabbits. However, all the biochemical parameters of the control batches obtained during this study are, with a few exceptions, consistent with those of [10].

At M1 and F1, there is an increase in both creatinine and urea values. According to [1, 19, 31, 28], an increase in serum urea concentration associated with creatinine would indicate renal failure. Therefore, it is possible to infer that males M1 and F1 would obey this

assertion. There is also an increase in triglycerides in M1 and M2, LDLc and potassium in M1. This rising triglyceride value would correspond to hypertriglyceridemia and would be a risk factor for predicting cardiovascular disease, according to [2, 12, 28]. This is because triglycerides are the main constituents of cell membranes and the increase in their concentration would probably be the result of apoptosis. Thus, M1 and M2 would obey this principle. As for the increase in LDLc in M1 and F2, it could favour the risk of coronary mortality because according to [24, 18, 28], its reduction would have the opposite effect. As for the transaminase elevation in F1, it could indicate hepatocellular damage or a disturbance of bile flow according to [11, 28]. Elevated ionogram values (Na, Cl and K) in F1, and blood glucose levels in F1 and M2 would indicate heart disease and diabetes respectively according to [30, 28]. In M2, the decrease in transaminases would reveal a vitamin B6 deficiency according to [5]. As for dyskalemia (potassium variation) in M1 and M2, it would be the basis of neuromuscular and cardiac signs according to [20]. The same author states that potassium plays a major role in the maintenance of the resting membrane potential. This observation was confirmed by [3] states that dyskalemia (hyperkalemia or hypokalemia) is life threatening. Thus, the various pathologies mentioned above would be linked to the action of venom on the vital organs of the various animals solicited, which would lead to their death.

CONCLUSION

This study, which aimed to understand the action of venom on the biochemical parameters of rabbits, revealed that:

- The venom of *Naja nigricollis* at a certain dose has a dose-dependent hemolysing power in vitro and in vivo;
- with a few exceptions, the biochemical parameters of males are generally higher than those of females;
- the venom of *Naja nigricollis* causes renal failure and cardiovascular disease in some animals, promotes a risk of coronary mortality and diabetes in others, causes neuromuscular and cardiac signs due to dyskalemia in others, and finally a hepatocellular damage or disruption of bile flow, a heart problem and vitamin B6 deficiency in one type of animal;
- The venom of *Naja nigricollis* is fatal.

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