

**CLINICAL AND HEMATOLOGICAL ALTERATIONS IN DOGS DURING  
EXPERIMENTAL ENVENOMATION WITH *Crotalus durissus terrificus* VENOM AND  
TREATED WITH ANTIOPHIDIC SERUM**

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**ABSTRACT:** The present work aimed to evaluate the clinical and hematological aspects during experimental envenomation by *Crotalus durissus terrificus* in dogs treated with different antiophidic serum doses. Sixteen dogs were divided into two groups of eight animals each. Group I received 1mg/kg venom subcutaneously and 30mg antiophidic serum intravenously; Group II received 1mg/kg venom subcutaneously and 60mg antiophidic serum intravenously. In the clinical evaluation, we observed ataxia, moderate sedation, dilated pupils, sialorrhea, flaccid paralysis of mandibular muscles, and discreet edema at the site of venom inoculation. Evaluating red and white blood cells, we observed a decrease of hemoglobins, globular volume and erythrocytes, and an increase of plasmatic proteins, leukocytes, neutrophils, monocytes and lymphocytes. Clotting time increased and there was blood incoagulability with return to normal clotting time six hours after antiophidic serum administration. Animals treated with six antiophidic serum flasks had a faster recovery than the animals that received three serum flasks.

**KEY WORDS:** *Crotalus durissus terrificus*, dog, clinical aspects, hematological aspects, venom.

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## **INTRODUCTION**

In Brazil, the genus *Crotalus* is considered of great importance due to the severe clinical aspects it provokes in humans and animals, and most of the time these can be fatal when treatment with specific serum is not quickly instituted (4). *Crotalus* snakes are considered more venomous than the snakes of the *Bothrops* genus (10). *Crotalus* venom is constituted of several fractions and this division reveals the following toxins: crotoxin, crotamine, giroxin, and convulxin (9, 20, 21, 22). Crotalic venom has three main actions: neurotoxic (12), myotoxic (5, 6), and hemolytic (23). The major complication observed in *Crotalus* accidents is the development of acute renal insufficiency with tubular necrosis (17, 23).

## **MATERIAL AND METHODS**

This experiment was approved by the Ethics Committee of University of Western São Paulo, UNOESTE, Presidente Prudente, São Paulo, Brazil.

We used sixteen dogs (eight females and eight males) between 1 and 4 years old, without a defined breed, weight varying from 6 to 15kg, from the Central Kennel of UNOESTE. By a complete physical exam and laboratory tests, animals that presented normal values were selected (18). They were kept in individual stalls in the kennel of the Veterinary Hospital of UNOESTE with water and food *ad libitum*.

The antiophidic serum (bothropic-crotalic) used was from Vencofarma Laboratory (serum sample 001/03); each flask of 10ml serum neutralizes 10mg crotalic venom.

Animals were divided into two groups with eight animals in each group, four females and four males, being Group I: animals inoculated with crotalic venom and treated with 30mg of antiophidic serum six hours after venom administration; and Group II: animals inoculated with crotalic venom and treated with 60mg of antiophidic serum six hours after venom administration.

Lyophilized crotalic venom donated from the Center for the Study of Venoms and Venomous Animals, CEVAP, UNESP, Brazil, was reconstituted in sterile saline solution and subcutaneously inoculated, at the dose of 1mg/kg live weight, into the animals' thigh lateral face; antiophidic serum was intravenously administered six hours after venom inoculation.

### **Clinical evaluation**

The following measurements: temperature, heart and breathing rate, pulse, pupil diameter, ataxia, sedation, and sialorrhea were obtained at different moments (Table 1).

### **Blood cell count**

Blood samples were collected by the jugular vein puncture using a hypodermic needle (30x08mm) and a syringe of 20ml; later, red blood cells and white blood cells were counted at different moments (Table 1).

### **Clotting Time**

Clotting time was evaluated at different moments by the Lee & White method [Table 1] (16).

### **Statistical Analysis**

Each variable was studied by using multivariate analysis, which allowed the comparison between groups at every moment as well as between moments of evaluation for each group.

For the variables whose evaluations were given by scores, groups were compared at every moment using the Kruskal-Wallis non-parametric test, and the effect of the moments on each group was compared by the Friedman non-parametric test (19, 28). A significance level of  $p < 0.05$  was adopted.

## **RESULTS AND DISCUSSION**

### **Clinical Evaluation**

Discreet edema was observed at the site of inoculation of crotalic venom (CV) in the animals of Groups I and II, which is in agreement with the data available in literature (7, 11).

Widespread muscular pain of moderate intensity was observed from 6 to 48 hours after venom inoculation and 42 hours after antiophidic serum administration on Groups I and II. Pain was evaluated by observing the dogs and graduated according to intensity (0 = no pain; 1 = light pain; 2 = moderate pain; 3 = serious pain).

Animals of Groups I and II presented ataxia and light sedation at M1 (4h after venom administration - AV) compared to the control moment (M0). This effect is resulting

from the CV action on the central and peripheral nervous systems (15, 25, 26). The animals were in lateral decubitus with moderate sedation 8 hours after CV inoculation and two hours after antiophidic serum administration and persisted until 24 hours AV and 18 hours after antiophidic serum administration with recovery beginning at M4 (48h AV) and completed at M6 (144h AV).

At M5 (72h AV), animals of Group II showed a faster recovery in relation to decubitus, ataxia and sedation, probably because they received a larger amount of antiophidic serum (60mg) compared to those of Group I, which received 30mg of the serum.

Evaluation of the temperature did not show any significant difference between moments for each group nor between groups for every moment. There was a decrease in the heart frequency of the animals of both groups at M1, although the values showed to be normal for this species. No alteration was observed in the pulse evaluation of the groups.

Regarding the breathing frequency, we observed just a small decrease in the animals of Group II at moments M1 and M2 (8h AV) compared to the other moments, and any significant alteration was verified compared to Group I.

Breathing alteration together with dyspnea, tachypnea, and acute breathing insufficiency was also mentioned by Amaral *et al.* (2) in three human patients victims of accident by *Crotalus durissus terrificus*. Signs appeared in the first 48 hours after the ophidic accident but the patients did not present alterations in radiographs of the thorax. In spite of the absence of significant breathing alterations in the animals of the present study, it is necessary to emphasize that complications due to paralysis of the breathing musculature are important complications mentioned by other authors (2, 12, 17).

According to a study with CV on bovine (29), the animals presented alterations in temperature parameters, and heart and breathing frequencies; however, these data are not pathognomonic to differentiate crotalic envenoming from another envenoming, for example bothropic.

Pupil diameter, normal at M0, started to become mydriatic from M1 to M5 in Group I and from M1 to M4 in Group II. Mydriasis occurred, being bilaterally responsive to light at all the moments studied (25). The pupil diameter in Group II returned to normality earlier than in Group I, probably due to a larger amount of antiophidic serum received by the former.

During the clinical evaluation, we noticed a discreet to moderate sialorrhea in the dogs of Group I from M1 to M3 (24h AV) and in those of Group II between M1 and M2. Again it was observed that Group II, probably as a result of the larger amount of antiophidic serum received (6 flasks), returned to normality in a smaller amount of time compared to Group I.

Besides sialorrhea clinical aspects, the animals of both groups used to stay with the mouth open, showing deglutition difficulty from M1 (4h AV) to M3 due to flaccid paralysis of the mandibular muscles. We observed vomit in five animals, three from Group I and two from Group II. Sialorrhea occurred due to the action of the venom neurotoxic fraction, a crotoxin that causes paralysis, similar to the effects caused by curare. Its main effect is neuromuscular transmission blockade, suggesting that paralysis and breathing alterations were due to the neuromuscular junction blockade (2). This fact allows for the conclusion that this neurotoxin acts in the presynaptic parasympathetic nerves, inhibiting acetylcholine release (2, 7).

According to Hudelson & Hudelson (13), vomit signs, salivation, defecation, and increase of the urinary frequency are linked to the autonomic nervous system involvement, however without confirmation.

### **Blood Cell Count**

As for the number of red blood cells, a significant decrease was observed ( $p < 0.05$ ) in Group II at M3 (72h AV) and M4 (144h AV) when compared to the control moment (M0), and a fast decrease was seen at M2 (24h AV) in relation to M0. In Group I, there was a fast decrease ( $p > 0.05$ ) of this parameter starting from M2 (Table 2).

Mean values of hemoglobin concentration and globular volume presented a significant fall ( $p < 0.05$ ) in relation to the control moment in dogs of Groups I and II, starting from M2 until M4 (Table 2).

Decrease of the variables erythrocyte, hemoglobin and globular volume can be attributed to the venom hemolytic action verified *in vitro* (25). Alteration in the serum coloration in blood samples collected from all the animals of Groups I and II six hours after venom inoculation was interpreted as hemolysis [Table 2] (27).

Increase of total plasmatic protein (TPP) was verified ( $p < 0.05$ ) for Group I at M3 in relation to the other moments. At that moment (M3), the protein plasmatic level in this group showed higher increase compared to Group II (Table 2). The high level of TPP can be explained by the clinical aspects of dehydration and the shock the animals

developed after CV inoculation (14), and also by the fact that the animals of Group II received a higher dose (60mg) of antiophidic serum. Differences were not observed in fibrinogen values in the comparison between groups at every moment and in the comparison between moments for each group. Studies have shown that the venom causes hypofibrinogenemia (1, 3, 15, 30); therefore, the non-alteration of the fibrinogen values might have been due to the technique used.

White blood cells from the animals of both groups showed leukocytosis with neutrophilia 24 hours after CV inoculation and 18 hours after serotherapy, returning to normal values in the subsequent moment (Table 3).

The monocytes number increased significantly at M3 in both groups, compared to the other moments; as for the number of lymphocytes, a decrease was observed in Groups I and II at M1 (6h AV) and M2 in relation to the control moment. Regarding eosinophil values, there was a decrease at M2 compared to M0 in Group I. In Group II, values remained close to those of M0 (Table 3).

Leukocytosis with neutrophilia can be due to interaction between CV and the animal organism, inducing a typical acute-phase response with release of catecholamines, cellular mediators and humor, besides serum chemotactic factors (7). Other possibility for leukocytic response would be the stress induced by pain, manipulation and discomfort of the animals leading to leukocytosis with neutrophilia, monocytosis, lymphopenia, and eosinopenia (14).

Clinical aspects of leukocytosis with a left shift displayed in the present study have been referred in the initial phase of crotalic envenoming in patients treated with specific serum (24). Jorge & Ribeiro (15) also verified leukocytosis aspects in eight patients bitten by rattlesnake, and six of them showed left shift, a response similar to that of one of the dogs in this study. Another report by Barraviera *et al.* (8) displays an elevation in the number of leukocytes and segmented neutrophils in crotalic accident with humans.

### **Clotting Time**

Evaluation of the clotting time showed a significant alteration from M1 (6h AV) for Groups I and II. The animals presented blood incoagulability six hours after CV inoculation. Six hours after the antiophidic serum administration, the clotting time returned to normality, and for Group I this return was more precocious than for Group II (Table 4). Alteration in the clotting time is due to the fraction named "thrombin-like",

capable of converting fibrinogen in fibrin, leading the patient to afibrinogenemia, which culminates with the prolonging of the clotting time (1, 3, 16, 30).

Table 1: Moments of evaluation of the clinical evolution, blood count and clotting time.

	M0	M1	M2	M3	M4	M5	M6
Clinical evaluation	Control	4h AV	8h AV	24h AV	48h AV	72h AV	144hAV
Blood count	Control	6h AV	24h AV	72h AV	144hAV	-	-
Clotting time	Control	6h AV	8h AV	10h AV	11h AV	12h AV	-

AV = after venom; h = hours. Antiophidic serum was administered 6h after venom inoculation.

Table 2: Means of red blood cell count according to groups and moments.

GROUPS	MOMENTS				
	M0	M1	M2	M3	M4
<b>Red blood cells (x10<sup>6</sup>/μl)</b>					
I	6,354	6,351Aa	5,896Aa	5,961Aa	5,634Aab
II	6,421	6,611Aa	6,288Aa	5,448Ba	4,889Ca
<b>Hemoglobin (g/dl)</b>					
I	15.04	15.57Aa	14.42ACa	13.67BCa	12.58Ba
II	15.72	16.66Aa	15.17Ba	13.75Ca	12.28Da
<b>Globular volume (%)</b>					
I	44.87	47.00Aa	43.25Ba	40.37BCa	38.12Cab
II	46.12	49.75Aa	45.12Ba	41.12Ca	36.37Da
<b>Total plasmatic protein (g/dl)</b>					
I	7.30	7.12Aa	7.02Aa	7.67Ba	6.97Aa
II	6.97	7.02Aa	6.95Aa	7.00Aab	6.67Aa

Capital letters compare moments in each group. Lowercase letters compare groups at every moment. Means followed by the same letters do not differ significantly ( $p>0.05$ ).

Table 3: Means of white blood cell count according to groups and moments.

<b>MOMENTS</b>					
<b>GROUPS</b>	<b>M0</b>	<b>M1</b>	<b>M2</b>	<b>M3</b>	<b>M4</b>
<b>Leucocytes/<math>\mu</math>l</b>					
<b>I</b>	11,950	16,350Aa	19,075Aa	15,920Aa	14,681Aa
<b>II</b>	10,610	13,470Aa	20,700Bb	14,910Aa	12,410Aa
<b>Segmented neutrophils (%)</b>					
<b>I</b>	7,417	12,973Aa	16,822Ba	11,351Aa	9,404Aa
<b>II</b>	6,045	11,611Aa	19,017Ba	9,204Aa	9,033Aa
<b>Monocytes (%)</b>					
<b>I</b>	496	780Aa	391Aa	1,376Ba	888Aa
<b>II</b>	532	367Aa	1026Aa	1135Bab	745Aa
<b>Lymphocytes (%)</b>					
<b>I</b>	2,635	1,793ABa	1,269Aa	2,335Ba	2,292Ba
<b>II</b>	2,600	1,849Aa	1,654Aab	2,529Aa	2,382Aa
<b>Eosinophils (<math>\mu</math>l)</b>					
<b>I</b>	1007Aa (668;2366)	701Aa (408;1281)	204Ba (144;297)	473Aa (348;1120)	1149Aa (651;1450)
<b>II</b>	515Aa (350;759)	290Ab (158;327)	0Aa (0;0)	328Aa (97;561)	341Ab (165;443)

Capital letters compare moments in each group. Lowercase letters compare groups at every moment. Means followed by the same letters do not differ significantly ( $p>0.05$ ).



Table 4: Means of clotting time according to groups and moments.

GROUPS	MOMENTS					
	M0	M1	M2	M3	M4	M5
	<b>Clotting Time</b>					
<b>I</b>	1.0 Aa (1.0;1.0)	0.0 Ba (0.0;0.0)	0.0 Ba (0.0;0.0)	0.0 Ba (0.0;0.0)	1.0 Aa (0.0;1.0)	1.0 Aa (1.0;1.0)
<b>II</b>	1.0 Aa (1.0;1.0)	0.0 Ba (0.0;0.0)	0.0 Ba (0.0;0.0)	0.0 Ba (0.0;0.0)	0.0 Ba (0.0;0.5)	1.0 Aa (1.0;1.0)

Capital letters compare moments in each group. Lowercase letters compare groups at every moment. Means followed by the same letters do not differ significantly ( $p>0.05$ ). Score: 0.0 = not clotted; 1.0 = clotted.

## CONCLUSION

Results of this experimental study with crotalic venom in dogs show that the venom causes important clinical alterations as ataxia followed by sedation, deglutition difficulty, sialorrhoea, midriasis, and muscular pain.

Hematological alterations were characterized mainly by the decrease in the erythrocytes number, hemoglobin and globular volume, and the increase in total plasmatic protein, besides leukocytosis with neutrophilia.

A picture of blood incoagulability was observed six hours after venom inoculation and clotting time returned to normality six hours after antiophidic serum administration. Hemolysis was observed in Groups I and II six hours after venom inoculation. Animals that received a larger amount of serum had a faster recovery.

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