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Clinical and laboratory evaluation of dogs experimentally intoxicated with toad venom

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ABSTRACT

This study aimed to evaluate the clinical and laboratory aspects of dogs intoxicated with toad poison. Overall, 20 male and female, adult healthy dogs of undefined breed were used and divided into two groups: control (n = 5) and intoxicated with toad venom (n = 15). The dogs were submitted to anesthesia induction with thiopental and maintained on inhalation anesthesia with 3% isoflurane during the evaluation and data collection period. In this period, animals in the control group received saline and the animals in the intoxicated group received an aliquot of toad venom through orogastric probe. Samples for hematological, serum biochemical and blood gas analyses were collected. During the evaluation, drooling, oral mucosa irritation, defecation, urination, congestion and hyperemia of the oral mucosa were observed. Intoxication due to bufotoxin caused evident gastroenteritis and neurological alterations with variable signs. Toad venom did not cause renal injury and may be considered non-nephrotoxic in the present experimental conditions. The intoxicated animals showed a slight liver change measured by the ALT and ALP values, and blood gas analysis showed that various acid-base balance changes may occur.

1. Introduction

Toads of the genus Rhinella (Bufo) produce a highly toxic venom, and although they cannot inoculate the poison, dogs become very common victims of poisoning by the secretion produced by toads when attacking them, causing the compression of glands that eliminate the venom, which will come into contact with the oral mucosa of dogs (Gadelha and Soto-Blanco, 2012, Gao et al., 2010, Jared and Antoniazzi, 2009). Dogs, especially puppies, are likely to play with these toads, and thus can get into contact with the secreted material and may suffer intoxication mainly at night (Macdonald, 1990, Roder, 2003). The absorption of toxins by the oral and gastric mucosa occurs rapidly (Macdonald, 1990, Palumbo and Perry, 1983).

The venom produced by toads, despite its complex structure, has two major groups of active substances: biogenic amines such as adrenalin, noradrenalin, serotonin, bufotenins and bufotionins and steroid derivatives such as bufodienolids and bufotoxins, which act similarly to digitalis causing inhibition of the sodium and potassium pump of cardiac muscle cells (Eubig, 2001 Gowda et al. 2,003, McFarland, 1999).

Dogs are the most common victims of poisoning due to bufotoxin because they are attracted by the slow movement of toads, and these are sometimes in the water supply of canines (Gadelha and Soto-Blanco, 2012). The clinical signs of intoxication due to toad venom can be divided into three groups according to the severity as mild, moderate or severe. In mild cases, the signals are only oral irritation and drooling. Moderate intoxication includes vomiting, depression, weakness, ataxia, neurological signs, heart rhythm abnormalities and spontaneous evacuation and urination. Severe cases include diarrhea, abdominal pain, sternal recumbency, pupils unresponsive to light, convulsion, pulmonary edema, cyanosis, and may progress to death (Camplesi et al., 2009; McFarland, 1999, Roberts et al., 2000).

The toad venom toxicity is variable and size- and dose-dependent. Toads have large parotoid glands and therefore great amount of venom (Sakate and Oliveira, 2000). Most clinical signs of severe poisoning occur in animals with low body weight that come into contact with large toads (Reeves, 2004; Roberts et al., 2000).

In a study by Roberts and Peterson (2001), neurological abnormalities were observed in over 50% of dogs intoxicated by toad venom. In this study, a moderate increase in hemoglobin, plasma urea and alkaline phosphatase was observed. The study also reported decrease in total protein and leukocytes, the latter mainly due to neutropenia, which was also reported by Palumbo and Perry (1983). Barbosa et al. (2009) reported two cases of toad venom intoxication in dogs, showing leukocytosis due to neutrophilia and one case with severe leukopenia. These authors suggest that changes in leukocyte counts are due to inflammation of the gastrointestinal tract, present in most intoxicated patients. Camplesi et al. (2010) observed neurological symptoms in all dogs experimentally intoxicated by toad venom.

In relation to serum biochemistry, Peterson and Roberts (2001) reported that alanine aminotransferase (ALT) and alkaline phosphatase (ALP) had no remarkable changes, but aspartate aminotransferase (AST) had an increase and then returned to normal values. Hyperkalemia is frequently observed both in dogs and humans intoxicated by toad venom, but hypokalemia was also reported (Camplesi et al., 2009; Eubig, 2001).

Toad venom intoxication is relatively common in dogs, resulting in variable and complex clinical condition that can be fatal. The rapid establishment of the clinical diagnosis is essential for appropriate treatment. Thus, this study aimed to evaluate the clinical and laboratory aspects of dogs intoxicated with toad poison in order to assist the small animal professional in establishing the diagnosis of this intoxication and the best therapy to be used in accordance with the clinical condition of the animal.

2. Materials and methods

The venom was collected from 20 toads of species Rhinella schineideri (formerly Bufo paracnemis), from the Herpetology Laboratory, Department of Zoology, Institute of Biosciences, UNESP, Botucatu, Brazil. The venom was extracted by manual compression of the parotoid glands. A pool of venom was stored in glass jars and sent to the Laboratory of Immunology, Department of Microbiology and Immunology, Institute of Biosciences, which was lyophilized in conventional lyophilizer at -30oC for three days. After this procedure, the venom was divided into

equal aliquots equivalent to 70% the amount of venom from one toad (approximately 0.1 g); such aliquots were placed in 20 mL test tube and stored at 5°C.

This study used 20 healthy dogs of undefined breed weighting approximately 10 kg each, from the Central Animal Laboratory, UNESP, Botucatu, Brazil. Were divided into control group (n = 5) and intoxicated group (n = 15). After a fasting period, the dogs were submitted to anesthetic induction with intravascular thiopental, 25mg/kg and anesthesia maintenance with 3% isoflurane. Throughout the anesthetic period, the dogs were kept in thermal mattress to control body temperature. Controlled ventilation was used in all dogs (FR = 10 to 15 mpm).

For the experimental poisoning, venom aliquots were resuspended in 10 mL of water and administered to each animal through an orogastric probe. Dogs in the control group received 10 mL of saline. Each animal remained anesthetized for 2,5 hours, and every 10 minutes, blood pressure, heart rate and rhythm were measured during the entire period that the animal remained anesthetized and later follow up to return to normal values. All signals shown were observed and recorded. During this period, the animals were maintained on intravenous fluid therapy with Ringer lactate.

Blood was collected from dogs at times T0 (before venom administration), T1 (two hours after venom administration), T2 (four hours after venom administration), T3 (six hours after venom administration), T4 (12 hours after venom administration) and T5 (24 hours after venom administration). Biochemical tests to evaluate possible kidney and liver damage (urea, creatinine, ALT, ALP, total protein - serum PT) were performed at T0, T3, T4 and T5. Blood gas analysis for measurement of plasma pH, partial O_2 pressure (PO_2), partial CO_2 pressure (PCO_2) and HCO_3, was held at all times, using arterial blood samples collected with heparinized syringe by puncturing the saphenous artery. Blood count was performed at times T0, T1 and T5.

Manual dilution in hematology chamber was performed for counts of erythrocytes and total leukocytes and differential count of leukocyte was performed in 100 cells in blood smears stained by panoptic method. Hemoglobin measurement used the cyanmethaemoglobin method with apparatus Celm SB-190. Plasma PT was measured by refractometry and packed cell volume (CV) by the microhematocrit method. Biochemical tests used commercial kits for urea, creatinine, ALT, ALP. The spectrophotometry method (colorimetric) was used to measure urea and the kinetic method was used to measure ALT, ALP and creatinine, both using the apparatus Celm SB-190 and with enzymatic activity at 37°C.

The statistical analysis used was ANOVA to compare groups, times and time x group interaction, followed by the Tukey's method when there was any significant effect. The significance level was 5%.

3. Results

During the period that they remained under anesthesia, the dogs in the control group showed no change in clinical signs, and returned to consciousness after the turn-off of the inhalation anesthesia apparatus. The body temperature of the animals was controlled with thermal mattress and kept around 37 to 38°C. The animals were observed up to 48 hours after anesthesia; all fed normally, remained in good general condition and had normal urine and feces in relation to the amount, appearance and coloring.

All dogs in the intoxicated group showed drooling in the immediate post-anesthesia period. Gastrointestinal signs were also evident three to six hours after intoxication, and 100% of dogs intoxicated with bufotoxin showed several emetic episodes with variable frequency (three to eight episodes), foamy and yellowish liquid content. In 40% of dogs (6/15), emesis was observed within 24 hours after venom administration. Pasty diarrhea of yellow-brown color was observed in 33% of animals (5/15) in the intoxicated group 48 hours after venom administration. This condition disappeared without any specific treatment. In 40% of dogs (6/15), congestion and hyperemia of the oral mucosa was observed.

All dogs in the intoxicated group showed neurological changes such as mydriasis, nystagmus, depression, tachypnea, and stupor. In 55% of dogs (8/15), spontaneous urination and/or defecation were observed during anesthesia. In 80% of animals (12/15), complete recovery was observed within 48 hours, but 20% (3/15) only obtained full recovery within 96 hours after venom administration, especially in relation to neurological signs.

The mean and standard deviation of erythrocytes, VC, hemoglobin, leukocytes and PT are detailed in Table 1. The counts of red blood cells of dogs in the control group ranged from 5.52 to 6.44 $x10^6/\mu L$, and all values are within the normal range for the species. However, dogs in the intoxicated group showed values between 3.00 and 8.14 RBC $x10^6/\mu L$. There was no statistically significant difference ($p > 0.05$) between times and groups. Although

there was no significant difference, 40% (6/15) of animals in the intoxicated group showed counts of red blood cells below the reference values for the species in accordance with Jain (1993) at time T5.

Table 1
Mean and standard deviation of values related to the counts of red blood cells / uL of blood, VG (%), hemoglobin (g / dL), white blood cells / uL of blood, PT (g / dL) according to time and group.

		Time			
Parameter	**Group**	**T0**	**T1**	**T5**	**Mean of group**
Red blood cells	Control	$5.53^{Aa}\pm11.33x10^{6}$	$5.65^{Aa}\pm0.15x10^{6}$	$6.28^{Aa}\pm0.54x10^{6}$	$5.82\pm0.45x10^{6}$
	Intoxicated	$5.75^{Aa}\pm0.47x10^{6}$	$5.41^{Aa}\pm1.20x10^{6}$	$5.60^{Aa}\pm1.27x10^{6}$	$5.59\pm1.03x10^{6}$
VG	Control	$40.00^{Aa}\pm5.70$	$36.80^{Aa}\pm1.10$	$41.00^{Aa}\pm4.10$	39.90 ± 4.50
	Intoxicated	$38.90^{Aa}\pm3.80$	$37.70^{Aa}\pm7.40$	$39.10^{Aa}\pm9.60$	38.60 ± 7.20
Hemoglobin	Control	$14.50^{Aa}\pm2.20$	$12.10^{Ab}\pm0.40$	$14.50^{Aa}\pm2.0$	13.70 ± 2.0
	Intoxicated	$13.00^{Aa}\pm1.60$	$13.50^{Aa}\pm1.70$	$14.00^{Aa}\pm2.3$	13.50 ± 1.9
Leucocytes	Control	$13.17^{Aa}\pm1.77x10^{3}$	$8.29^{Ab}\pm1.69x10^{3}$	$16.42^{Aa}\pm0.93x10^{3}$	$12.63\pm3.73x10^{3}$
	Intoxicated	$7.58^{Bb}\pm1.92x10^{3}$	$7.30^{Ab}\pm2.32x10^{3}$	$12.42^{Ba}\pm4.34x10^{3}$	$9.10\pm3.81x10^{3}$
PT	Control	$6.50^{Aa}\pm0.40$	$6.20^{Aa}\pm0.00$	$6.10^{Aa}\pm0.10$	6.30 ± 0.30
	Intoxicated	$6.50^{Aa}\pm0.60$	$6.20^{Aa}\pm1.40$	$6.80^{Aa}\pm1.40$	6.50 ± 1.20

$p<0.05$. Lowercase letters compare mean of times in each group. Capital letters compare means of groups at each time. Same letter do not differ.

The VG values of dogs in the control group ranged from 35 to 51%, and all were within the normal range for the species in accordance with references values proposed by Jain (1993). Dogs in the intoxicated group showed VG values between 20 and 57%. There was no statistically significant difference ($p> 0.05$) between groups or times within each group. Although there was no significant difference, 26% of animals in this group (4/15) had VG values below reference values at times T1 and T5.

The hemoglobin values of dogs in the control group ranged from 12.0 to 17.8 g/dL, and were within the normal range for the species according to reference values proposed by Jain (1993). The animals in the intoxicated group had hemoglobin levels between 12 and 20.1 g/dL. There was no statistically significant difference between groups. A significant difference ($p <0.05$) between times in the control group, and time T1 showed lower values when compared with the other two times.

The leukocyte values of dogs in the control group ranged from 6 to $17x10^{3}/\mu L$, and are within the normal range for the species according to reference values proposed by Jain (1993). However, dogs in the intoxicated group showed leukocyte values ranging from 3990 to 18.69 $x10^{3}/\mu L$. In comparison between groups, there was no significant difference between times T0 and T5, and the intoxicated group showed values significantly lower than the control group. Among the times within the control group, there was no statistical difference in time T1 compared with the other two times; the intoxicated group showed significant difference in time T5, compared to the other times.

In the differential analysis of leukocytes in relation to neutrophils, there was no statistical difference; however, at time T1, 46% of animals (7/15) in the intoxicated group showed neutrophil values below the normal range for the species (Jain, 1993) and 6% of dogs (1/15) in the intoxicated group had values above the normal range for the species. At time T5, 13% of animals (2/15) in the intoxicated group showed values above the normal range for the species, according to reference values. In relation to lymphocytes, no significant difference was observed; however, at time T1, 20% of animals (3/15) in the intoxicated group had lymphocyte values below the normal range for the species and 20% of dogs (3/15) in the intoxicated group had values above the normal range for the species. At time T5, 13% of animals (2/15) in the intoxicated group had values above the normal range for the species. In the monocytesm count, no significant difference was observed; however, at time T1, 60% of animals (9/15) in the intoxicated group showed monocyte values above the normal range for the species. At time T5, 33% of animals (5/15) in the intoxicated group had values above the normal range.

The PT values of dogs in the control group ranged from 6.0 to 6.9 g/dL, and are within the normal range for the species according to reference values proposed by Jain (1993). Dogs in the intoxicated group showed PT values between 5 and 11.4 g/dL. There was no statistically significant difference ($p> 0.05$) between groups or times within each group.

Although there was no significant difference, 46% of animals (7/15) in the intoxicated group had PT values below the normal range for the species at time T1 and 6% of animals (1/15) in this group showed values above the normal range for the species at time T1. At the time T5, 13% of animals (2/15) had values below the normal range for the species and 6% of animals (1/15) in this group had value above the normal range (Jain, 1993).

The mean and standard deviation of urea, creatinine, ALT and ALP values are detailed in Table 2. The urea levels of dogs in the control group ranged from 21 to 46mg/dL, and are within the normal range for the species according to reference values proposed by Kaneko et al. (1997). Dogs in the intoxicated group showed urea levels between 17 and 48mg/dL. There was no statistically significant difference ($p > 0.05$) between groups or times within each group. Although there was no significant difference in time T4, 6% of animals (1/15) in the intoxicated group showed value below the normal range for the species. At time T5, 20% of animals (3/15) had values below the normal range.

Table 2
Mean and standard deviation related to urea (mg / dL), creatinine (mg / dL), ALT (IU / L) and ALP values (IU / L) according to time and group.

		Time				
Parameter	**Group**	**T0**	**T3**	**T4**	**T5**	**Mean of group**
Urea	Control	23.40±2.90	31.00±7.10	36.00±9.70	32.40±4.00	30.70±7.60
	Intoxicated	26.90±4.90	32.90±7.90	33.70±9.10	30.40±8.50	31.00±8.00
Creatinine	Control	0.70±0.10	0.60±0.10	0.80±0.20	0.70±0.10	0.70±0.10
	Intoxicated	0.70±0.10	0.70±0.10	0.60±0.10	0.70±0.10	0.70±0.10
ALT	Control	23.50±3.10	26.70±1.10	40.80±11.80	39.30±6.30	32.60±10.00
	Intoxicated	23.20±5.30	40.40±22.10	39.60±20.90	43.40±22.80	36.60±20.30
ALP	Control	71.70±11.30	105.60±52.00	80.70±29.80	81.80±35.20	75.40±24.90
	Intoxicated	79.80±40.30	125.00±64.30	125.00±64.30	159.80±80.90	117.50±66.6

The creatinine values of dogs in the control group ranged from 0.5 to 1.2 mg/dL, and are within the normal range for the species according to reference values proposed by Kaneko et al. (1997). Dogs in the intoxicated group showed creatinine values between 0.4 and 0.9 mg/dL. There was no statistically significant difference ($p > 0.05$) between groups or times within each group.

The ALT levels of dogs in the control group ranged from 19.4 to 40.8 IU/L and are within the normal range for the species according to reference values proposed by Kaneko et al. (1997). Dogs in the intoxicated group showed ALT values between 13.1 and 87.5 IU/L. There was no statistically significant difference ($p > 0.05$) between groups or between times within each group. Although not significantly different, at time T3, 13% of animals (2/15) in the intoxicated group had values above the normal range for the species. At time T4, 20% of animals (3/15) had values above the normal range for the species and at time T5, 26% of dogs (4/15) had values above the normal range.

The ALP values of dogs in the control group ranged from 33.2 to 131.5 IU/L, and are within the normal range for the species according to reference values proposed by Kaneko et al. (1997). However, dogs in the intoxicated group showed ALP values between 33.2 and 306.2 IU/L. There was no statistically significant difference ($p > 0.05$) between groups or times within each group. Although not significantly different, at time T3, 13% of animals (2/15) in the intoxicated group had values above the normal range for the species. At time T4, 33% of dogs (5/15) in this group had values above the normal range for the species and at time T5, 53% of animals (8/15) in this group had values above the normal range.

The mean and standard deviation of pH, HCO_3, PO_2 and PCO_2 obtained in the blood gas measurement are shown in table 3. The blood pH values in the control group ranged from 7.35 to 7.45, and are within the normal values for dogs according to Di Bartola (2005). In the intoxicated group, blood pH values ranged from 7.27 to 7.54. There was no statistically significant difference ($p > 0.05$) between groups. There was a significant difference ($p < 0.05$) between times in the intoxicated group, and at time T5, this group showed higher values compared to the other times. Although there was no statistical difference between groups, the animals in the intoxicated group had values below the reference values for the species in 13% of animals (2/15) at time T0, 46% of animals (7/15) in T1,

33% of animals (5/15) in T2 and 26% of animals (4/15) in T3, and values above the normal range were observed in 13% of animals (2/15) at T0, 6% of dogs (1/15) in T1, 13% of animals (2/15) in T3 and 53% of dogs (8/15) in T4.

In the control group, the plasma bicarbonate values ranged from 18.2 to 23.8 mmol/L and were within the normal range for dogs according to Di Bartola (2005). In the intoxicated group, the plasma bicarbonate values ranged from 14.7 to 25.3 mmol/L. There was no statistically significant difference ($p > 0.05$) between groups. There was a significant difference ($p < 0.05$) between times in the intoxicated group. Although there was no difference between groups, the animals in the intoxicated group had values below reference for the species in 6% of animals (1/15) in T1, 13% of animals (2/15) in T2, 20 % of dogs (3/15) in T3 and in 6% of animals (1/15) in T4.

Plasma PO_2 values in the control group ranged from 83.6 to 485.6 mmHg, and were within the reference values for the species according to Di Bartola (2005). In the intoxicated group, plasma PO_2 values ranged from 52.1 to 500.8 mmHg, being below the reference values for the species in 13% of dogs (2/15) in T1, 26 % of animals (4/15) in T2, 26% of dogs (4/15) in T3 and 26% of animals (4/15) in T4. There was no statistically significant difference ($p > 0.05$) between groups. There was a significant difference ($p < 0.05$) between times when the overall averages of times were compared, and times T2, T3 and T4 differed from times T0 and T1.

The plasma PCO_2 values in the control group ranged from 24.3 to 44.3 mmHg in 60% of dogs (3/5) at time T0 and 20% of animals (1/5) at time T1 showed values higher than reference for the species and 20% (1/5) at time T2 had values below reference according to Di Bartola (2005). In the intoxicated group, the PCO_2 values ranged from 22.4 to 51.2 mmHg, being above the reference values for the species in 33% of dogs (5/15) at T0, 53% of dogs (8/15) at T1, and 40% of animals (6/15) at T2 and 13% of dogs (2/15) at time T3 had values below the reference values, 6% of dogs (1/15) at T3 and 20% of animals (3/15) at T4. There was no statistically significant difference ($p > 0.05$) between groups. There was a significant difference ($p < 0.05$) between times within the control group at T0 and T2, and between times T3 and T4 in the intoxicated group.

4. Discussion

The selection of the genus Bufo was based on the importance of accidents caused by this amphibian. Toads of this genus are common in Brazil due to optimal conditions for their survival. Thus, toad poisoning is a serious problem for animal and human health (Chi et al., 1998; Sakate and Oliveira, 2000; Eubig, 2001). Animals in the intoxicated group showed several clinical signs consistent with poisoning due to bufotoxin. The signals were mainly of gastrointestinal and neurological origins.

The gastrointestinal signs presented by dogs intoxicated with bufotoxin were drooling, hyperemia and congestion of the oral mucosa, frequent vomiting of yellowish content and pasty diarrhea. These signs disappeared within 48 hours after administration of Bufo schineideri toad venom without specific treatment. These results are consistent with those described by Knowles (1968), Otani et al. (1969) Bedford (1974), Palumbo and Perry (1983) McFarland (1999) and Camplesi (2006).

The neurological signs were quite evident and were present in 100% of intoxicated animals in this work, with variable signs. The neurological signs most commonly observed were unresponsive mydriasis, nystagmus, stupor, tachypnea, opisthotonus and ataxia. Since the animals were anaesthetized and remained under anesthesia for two hours, the neurological signs were only observed after the anesthetic period. The signals remained up to 96 hours after venom administration in some animals. The data found in this study agree with those described in literature (Knowles, 1968; Otani et al., 1969; Bedford, 1974; Palumbo and Perry, 1983; Macdonald, 1990; McFarland, 1999).

The variation in both severity and type of clinical signs is due to several factors such as toad species, venom power, individual susceptibility, among others (Knowles, 1968; Otani et al. 1969; Oehme et al. 1980; Eubig, 2001; Camplesi, 2006).

The erythrocyte evaluation showed reduction of red blood cell count in 53% of dogs in the intoxicated group and packed cell volume in 40% of dogs in the intoxicated group, possibly due to anorexia presented by these dogs after intoxication and to the occurrence of gastroenteritis, which was present in all animals of this group. The decrease in packed cell volume is possibly due to the fluid therapy to which the animals were submitted during the experiment. There is no data in literature to support a discussion on the topic.

The increase in total plasma protein, which was observed in 6% of dogs in the intoxicated group was possibly a result of hypovolemia (dehydration) due to the occurrence of water loss as emesis and pasty defecation, as a result of gastroenteritis caused by toad venom. Increased total serum protein is a laboratory finding associated with dehydration (Rebar et al., 2003). In 26% of dogs in the intoxicated group, decreased total serum protein was

also observed. This decrease may be due to the pre-anesthetic 12 hours fasting period to which the animals were submitted and to the occurrence of hypo/anorexia after experimental intoxication. The decrease in total plasma protein is described in literature by Russell (1979), Palumbo and Perry (1983) and Peterson and Roberts (2001), in agreement with the results found in this experiment.

In the white blood count evaluation, the occurrence of leukopenia due to neutropenia in approximately 40% of dogs in the intoxicated group was observed. These findings agree with data from Russell (1979), Palumbo and Perry (1983) and Peterson and Roberts (2001), who found severe neutropenia in dogs experimentally intoxicated with toad venom. The occurrence of lymphopenia in 26% of dogs and monocyte in 60% of dogs in the intoxicated group was also observed. The changes found in white blood count of some intoxicated dogs are compatible with stress white blood count, common in cases where there is release of endogenous cortisol. Ferreira Neto et al. (1981) described the destruction of blood cells by adrenocortical hormones and also report that relative monocytosis may occur in leukopenia and neutropenia.

The urea value below reference values according to Kaneko et al. (1997) found in 20% of dogs in the intoxicated group is possibly due to the low protein intake, the pre-anesthetic fasting period and anorexia of dogs after intoxication. Low or marginal dietary protein levels lead to reduced plasma urea concentrations (Kerr, 2003).

Creatinine values within the reference values and the urea values that were only found in some dogs below the reference values according to Kaneko et al. (1997) suggest that bufotoxin causes no change in renal function and can be considered non nephrotoxic.

Slightly elevated ALT enzyme values were found in 33% of dogs in the intoxicated group and this is possibly due to the hepatic metabolization of bufotoxin (Ettinger and Feldman, 1997, Kerr, 2003). Slightly elevated ALP values in 46% of dogs in the intoxicated group are possibly due to thiopental. Some drugs can stimulate the production of certain enzymes; in particular, barbiturates may increase the activity of ALP and ALT (Kerr, 2003).

In the evaluation of blood pH, dogs in the control group showed values within the normal range for the species in accordance with Di Bartola (2005). However, animals in the intoxicated group showed both a decrease in pH, most evident at times T1, T2 and T3, as an increase in this parameter, more evident at time T4. The decreased blood pH is a common alteration in cases of poisoning with bufotoxin, and most often is of metabolic origin, caused by decreased blood bicarbonate (Ettinger and Feldman, 1997).

Carbon dioxide is considered potentially acid, due to its ability to bind to water and produce carbonic acid (Di Bartola, 2005). Thus, the results obtained in the three first times of this experiment are in agreement with literature, since metabolic and respiratory acidosis was observed. The pH elevation, which occurred at the last time, was possibly caused due to compensatory respiratory alkalosis, which aimed to correct acidosis, both metabolic and respiratory due to the effects of toad venom.

Regarding plasma bicarbonate, animals in the control group had values within the normal range for the species according to Di Bartola (2005). In animals in the intoxicated group, a decrease in the bicarbonate values was observed, and 20% of dogs had values below the reference for the species. According to the results found in this study, the occurrence of metabolic acidosis in animals in the intoxicated group was observed. These results are in agreement with literature, which describes that metabolic acidosis is common in cases of poisoning (Ettinger and Feldman, 1997).

The PCO_2 values were high in 20% of dogs in the control group and in 86% of dogs in the intoxicated group, which shows a condition of significant hypoventilation, which led to the accumulation of CO_2, featuring respiratory acidosis. This hypoventilation led to reduced PO_2 values, which may cause tissue hypoxia. Regarding PO_2, in dogs in the control group, all results were within the normal range, according to Di Bartola (2005). About 60% of animals in the intoxicated group had values below the normal range for the species. Hypoventilation in these animals was possibly due to respiratory depression caused by anesthesia, including transient apnea following thiopental administration, which occurred in some animals (Massone, 1994).

In contrast, decreased PCO_2 was observed in 60% of animals in the control group and 26% of animals in the intoxicated group. This decrease is due to hyperventilation, which may have been caused by the organism in the attempt to compensate for the metabolic acidosis, causing a compensating respiratory alkalosis. Furthermore, this change in respiratory rate may have been caused by the effect of venom components (bufotenines and bufotionins) on the central nervous system, stimulating the bulbar center, which leads to an increase in respiratory rate (Chen and Kovarikova, 1967; Monti and Cardello, 1994). In animals in the control group, hyperventilation occurred possibly as a compensatory mechanism of the body after transient apnea caused by the administration of sodium thiopental used for intravenous anesthesia induction.

Table 3
Mean and standard deviation related to pH, HCO_3 and PO_2 (mmHg), PCO_2 (mmHg) and according to time and group

		Time					
	Group	**T0**	**T1**	**T2**	**T3**	**T4**	**Mean of group**
pH	Control	7.40^{b}±0.00	7.40^{b}±0.00	7.40^{b}±0.00	7.40^{b}±0.00	7.40^{b}±0.00	7.40±0.00
	Intoxicated	7.40^{b}±0.01	7.40^{b}±0.01	7.40^{b}±0.00	7.40^{b}±0.01	7.50^{a}±0.00	7.40±0.01
HCO_3	Control	21.00^{a}±2.10	20.40^{a}±1.80	20.50^{a}±2.10	19.70^{a}±1.00	21.50^{a}±0.80	20.60±1.60
	Intoxicated	22.20^{a}±1.70	21.10^{ab}±2.10	19.80^{bc}±3.0	18.70^{c}±2.20	19.50^{bc}±1.9	20.20±2.50
PO_2	Control	426.10^{a}±43.3	409.30^{a}±49.5	98.88^{a}±8.80	90.60^{a}±4.70	95.10^{a}±69.0	224.00±163.8
	Intoxicated	435.40^{a}±44.3	410.50^{a}±38.7	87.40^{b}±14.0	88.80^{b}±12.7	90.20^{b}±12.3	217.00±166.0
PCO_2	Control	38.80^{a}±3.80	35.20^{ab}±3.10	28.60^{b}±3.30	30.00^{ab}±1.5	33.70^{ab}±2.7	33.30±4.60
	Intoxicated	36.30^{a}±3.60	39.60^{a}±7.10	35.40^{a}±6.10	31.80^{bc}±5.0	28.90^{c}±3.80	34.40±6.40

$p<0.05$. Lowercase letters compare mean of times in each group. Same letter do not differ.

5. Conclusion

Thus, it could be concluded that in toad venom intoxication, the neurological signs are present and variable, and there is a clear gastroenteritis, the observed changes in erythrocyte evaluation are nonspecific, and there are several changes in the acid-base balance. Toad venom causes no kidney damage, and may be considered non-nephrotoxic, but caused liver disorders measured by the ALT and ALP values in the present experimental conditions.

Conflict of Interest Statement

There are no conflicts of interest.

References

Barbosa, C.M., Medeiros, M.S., Riani Costa, C.C.M., Camplesi, A.C., Sakate, M., 2009. Toad poisoning in three dogs: case reports. J. Venom Anim.Toxins Incl. Trop. Dis., 15, 789-798.

Bedford, P.G.C., 1974. Toad venom toxicity and its clinical occurrence in small animals in the United Kingdom. Vet. Rec., 94, 613-614.

Camplesi, A.C., 2006. Intoxicação experimental por veneno de sapo: estudos clínico, laboratorial, eletrocardiográfico e da resposta ao tratamento com propranolol em cães. 102f. Dissertação (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu.

Camplesi, A.C., Sakate, M., Simão, N.M.B., Moya, C.F., 2009. Dosagem de marcadores cardíacos CK-MB e TnIc e eletrólitos no envenenamento experimental por veneno de sapo em cães. Pesq. Vet. Bras., 29, 632-636.

Camplesi, A.C., Sakate, M., Simão, N.M.B., Marucio, R., Mota, F.C.D., Moya-Araujo, C.F., 2010. Clinical and electrocardiographic evaluation during experimental toad poisoning in dogs. J. Venom Anim.Toxins Incl. Trop. Dis., 16, 342-354.

Chen, K.K., Kovarikova, A., 1967. Pharmacology and toxicology of toad venom. Pharmacol. Exp. Ther., 56, 1535-1541.

Chi, H.T., Hung, D.Z., Hu, W.H., Yang, D.Y., 1998 Prognostic implication of hiperkalemia in toad toxin intoxication. Human Exp. Toxicol., 17, 343-346.

Di Bartola, S.P., 2005. Fluid Therapy in Small Animal Practice. 2nd. ed. W. E. Saunders, Philadelphia. 611p.

Ettinger, S.J., Feldman, E.C., 1997. Tratado de Medicina Interna Veterinária. 4th. ed. Manole, São Paulo, 1983p.

Ferreira Neto, J.M., Viana, E.S., and Magalhães, L.M., 1981. Patalogia Clínica Veterinária. 2nd. ed., Gráfica Rabelo, Belo Horizonte, 292p.

Eubig, P.A., 2001. Bufo species toxicosis: Big toad, big problem. Vet. Med. 595-599.

Gadelha, I.C.N., Soto-Blanco, B., 2012. Intoxicação de cães por sapos do gênero Rhinella (Bufo) – revisão de literatura. Clínica Veterinária. 100, 46-52.

Gao, H., Zehl, M., Leitner, A., Wu, X., Wang, Z., Koop, B., 2010. Comparison of toad venoms from different Bufo species by HPLC and LCDAD-MS/MS. J. Ethnopharmacol., 131, 368-376.

Gowda, R.M., Cohen, R.A., Khan, I.A., 2003. Toad venom poisoning: resemblance to digoxin toxicity and therapeutic implications. Heart., 14, 483-485.

Jain, N.C., 1993. Essentials of veterinary hematology, Lea & Febinger, Philadelphia. 417p.

Jared, C., Antoniazzi, M.M., 2009. Anfíbios: biologia e venenos. In Animais peçonhentos no Brasil: biologia, clínica e terapêutica dos acidentes (J.L. Cardoso, F.O.S. França, F.H. Wen, C.M.S. Málaque, and V. Haddad JR., Eds.), 2nd ed., pp.317-330, Sarvier, São Paulo, SP.

Kaneko, J.J., Harvey, J.W., and Bruss, M.L., 1997. Clinical biochemistry of domestic animals, 5nd. ed. Academic Press, San Diego. 932p.

Kerr, M.G., 2003. Exames Laboratoriais em Medicina Veterinária Bioquímica Clínica e Hematologia. 2nd. ed. Roca, São Paulo, 436p.

Knowles, R.P., 1964. The poison toad and the canine. Vet. Med. Small Anim. Clin., 39-42.

Macdonald, B., 1990. Terrier toad toxicity syndrome. Austr. Vet. Practit., 20, 118-120.

Massone, F., 1994. Anestesiologia veterinária-farmacologia e técnicas. 2nd. ed. Guanabara Koogan, Rio de Janeiro, 252p.

Mcfarland, P.J., 1999. Toad Toxicity. Aust. Vet. Pract., 29, 98-103.

Monti, R., Cardelo, L., 1994. Bioquímica do veneno de anfíbios. In Venenos animais: uma visão integrada (B. Barra Viera, Eds.). pp.225-232, EPUC, Rio de Janeiro.

Oehme, F.W., Brown, J.F., Fowler, M.E., 1980. Toxins of animal origin. In Toxicology: the basic science of poisons. (L.J. Casarett, and J. Doull, Eds.) 2nd. ed. MacMillan, New York, 568p.

Otani, A., Palumbo, N., Read, G., 1969. Pharmacodynamics and treatment of mammals poisoned by Bufo marinus toxin. Am. J. Vet. Res., 30, 1865-72.

Palumbo, N.E., Perry, S.F., 1983. Toad poisoning. In Current Veterinary Therapy: Small Animal Practice (R.W., Kirk, Eds). 8nd. ed., pp.160-162, W.B. Saunders, Philadelphia.

Peterson, M.E., Roberts, B.K., 2001. Amphibian toxins. In Small Animal Toxicology. (M.E. Peterson and P.A. Talcott, Eds), pp.396-404. W.B. Saunders, Philadelphia.

Rebar, A.H., MacWilliams, P.S., Feldman, B.F., Metzger, F.L., Pollock, R.V.H., Roche, J., 2003. Guia de hematologia para cães e gatos. 1rst. ed. Roca, São Paulo., 245p.

Russel, F.E., 1979. Toad poisoning. In Canine medice. (H.P. Hoskins, Eds) 4th. ed. pp.183-185, American Veterinary Publications Incorporation, Santa Barbara.

Reeves, M.P., 2004. A retrospective report of 90 dogs with suspected cane toad (Bufo marinus) toxicity. Aust. Vet. J., 82, 608-611.

Roberts, B.K., Aronsohn, M.G., Moses, B.L., Burk, R.L., Toll, J., Weeren, F.R., 2000 Bufo marinus intoxication in dogs: 94 cases (1997-1998). J. Am. Vet. Med. Assoc., 216, 1941-4.

Roder, J.D., 2003. Toads. In Clinical Veterinary Toxicology (K.H. Plumlee, Eds), pp. 85-93, Mosby, St. Louis.

Sakate, M., Oliveira, P.C.L., 2000. Toad envenoming in dogs: Effects and treatment. J. Venom Anim. Toxins., 6, 52-62.