

## ANTIVENOM ACTION ON RENAL EFFECTS INDUCED BY *Thalassophryne nattereri* VENOM

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**ABSTRACT:** *Thalassophryne nattereri* (niquim) is a venomous fish responsible for numerous accidents involving fishermen in northern and northeastern Brazil. The aim of the present investigation was to evaluate the action of antivenom on renal effects caused by *Thalassophryne nattereri* venom. Isolated kidneys of Wistar rats were perfused with a previously dialyzed Krebs-Henseleit solution containing 6 g% bovine serum albumin. The antivenom action was studied through perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF) and glomerular filtration rate (GFR). The niquim venom (1 µg/mL), the antivenom alone (1 µg/mL) or the venom incubated with antivenom were added to the system 30 minutes after the beginning of each perfusion. Previous works have shown venom induced-alterations of renal function parameters. In the isolated rat kidney, *T. nattereri* venom (1 µg/mL) increased the perfusion pressure and renal vascular resistance at 60, 90 and 120 minutes. UF and GFR also increased at 60, 90 and 120 minutes when compared with the control group; however, no effects were observed on the percent of sodium (%TNa<sup>+</sup><sub>control</sub> = 81.1 ± 0.86; %TNa<sup>+</sup><sub>60</sub> = 78.04 ± 1.18; %TNa<sup>+</sup><sub>90</sub> = 76.16 ± 3.34; %TNa<sup>+</sup><sub>120</sub> = 79.49 ± 0.87) and potassium (%TK<sup>+</sup><sub>control</sub> = 72.29 ± 1.12; %TK<sup>+</sup><sub>60</sub> = 75.41 ± 0.65; %TK<sup>+</sup><sub>90</sub> = 71.23 ± 2.55; %TK<sup>+</sup><sub>120</sub> = 76.62 ± 1.04) tubular transport. The administration of the antivenom (1 µg/mL) incubated with venom (1 µg/mL) reduced the changes in PP, RVR, UF and GFR provoked by *Thalassophryne nattereri* venom. The group perfused with venom alone showed a moderate deposit of a proteinaceous material in the tubules and urinary space. The group perfused with the antivenom presented similar results to the control group. In conclusion, the antivenom was able to decrease the effects induced by *T. nattereri* venom in isolated rat kidney.

**KEY WORDS:** *Thalassophryne nattereri*, antivenom, kidney.

**CONFLICTS OF INTEREST:** There is no conflict.

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## INTRODUCTION

*Thalassophryne nattereri*, also known as niquim, is a venomous fish responsible for several accidents among fishermen in northern and northeastern Brazil (1-3). On coast of Ceará State, from 1992 to 2002, 16 envenomation cases by *T. nattereri* were registered (4). The number of injuries is not so small considering an active study on envenomation frequency that shows a very high incidence in all fisherman communities from northeast region of Brazil. The *Thalassophryne* bite causes pain, edema, erythema and occasional necrosis, which are also seen in highly necrotic venoms, as the one from freshwater stingrays (2).

*T. nattereri* venom is composed of proteolytic and myotoxic toxins devoid of phospholipase A<sub>2</sub> activity (5). Analysis of skeletal muscles of mice injected with the venom revealed damage to muscles (6). Some studies demonstrated that the action of the venom on endothelium may contribute to ischemia (7). On isolated rat kidney we observed that *T. nattereri* venom causes changes in renal function including increase in: perfusion pressure, renal vascular resistance, urinary flow and glomerular filtration rate (8). Local acute inflammatory response induced by the venom was also registered (9). Cytokines, as TNF- $\alpha$ , IL-1 $\beta$  and IL-6, and a weak leukocyte influx were detected on footpads of mice. Additionally, a cytotoxic effect of the venom on mononuclear cells was recorded. *T. nattereri* venom content stimulates an association of cytokines of both Th1 and Th2 profile, with a notable IL-5 production and secretion of specific IgG1 and total IgE isotypes, which may also affect B cells and induce a memory antibody response by the secretion of protective IgG subclasses (10).

Anti-inflammatory drugs, like dexamethasone and indomethacin, are inefficient to control envenomation symptoms (11). It was demonstrated that harmful effects of *T. nattereri* envenomation on mice could be prevented by previous long-lasting immunization (12). Since systemic antibody levels could be induced by this venom and protect mice against venom deleterious properties, we therefore tested the capacity of antibodies present in horse serum to neutralizing or reducing renal effects induced by the fish venom.

## **MATERIALS AND METHODS**

### **Venom**

Specimens of adult *T. nattereri* fish were collected in the Brazilian state of Alagoas. The venom was obtained according to the technique described by Lopes-Ferreira *et al.* (5). Protein concentrations were determined through a colorimetric method (13). Standard curves were constructed using bovine serum albumin (Sigma Chemicals, USA) diluted in duplicate.

### **Serum Antivenom of *T. nattereri***

Hyperimmune serum was obtained from horses injected with *T. nattereri* venom (12). Each animal received 26 mg of venom during the process.

### **Kidney Perfusion**

Adult male Wistar rats (260 to 320 g) were fasted for 24 hours with free access to water. The animals were anesthetized with sodium pentobarbitone (50 mg/kg, IP). After careful dissection of the right kidney, the right renal artery was cannulated via the mesenteric artery without interruption of blood flow (14, 15). The perfusate consisted of a modified Krebs-Henseleit solution (MKHS) containing 118.0 NaCl; 1.2 KCl; 1.18 KH<sub>2</sub>PO<sub>4</sub>; 1.18 MgSO<sub>4</sub>.7H<sub>2</sub>O; 2.50 CaCl<sub>2</sub> and 25.0 NaHCO<sub>3</sub> in mmoles/L. Six grams of bovine serum albumin (BSA) were added to 100 mL of MKHS, and dialyzed for 48 hours at 4°C against ten volumes of MKHS. Immediately before the beginning of each perfusion protocol, 100 mg of urea, 50 mg of inulin and 50 mg of glucose were added to every 100 mL of perfusate and pH was adjusted to 7.4. Each experiment lasted 120 minutes and the recirculating perfusion system employed 100 mL of MKHS.

Perfusion pressure (PP) was measured at the tip of the stainless steel cannula in the renal artery. Samples of urine and perfusate were collected at 10-minute intervals for analysis of sodium and potassium level by flame photometry; inulin was determined by direct hydrolysis and osmolality was measured in a vapor pressure osmometer (Wescor 5100C, USA) (16). The *Thalassophryne nattereri* venom and antivenom were preincubated at 37°C for 30 minutes before addition in perfusion system. The venom (1 µg/mL), the antivenom alone (1 µg/mL) or venom incubated with antivenom were added to the system 30 minutes after the beginning of each perfusion. Renal vascular resistance (RVR), urinary flow (UF) and glomerular filtration rate (GFR) were

determined (17). Results were compared to a control group where the kidneys were perfused with Krebs-Henseleit solution alone. Statistical analysis was performed by means of one-way ANOVA and Bonferroni t-test with the level of significance set at  $p < 0.05$  and  $n = 6$  to each group.

### **Renal Histological Evaluation**

After the renal perfusion experiment, both right and left kidneys were removed and fixed in 10% formaldehyde for histological processing. Kidney tissue was embedded in paraffin, cut into 3- to 5- $\mu\text{m}$  sections, stained with hematoxylin-eosin and further processed for light microscopy (Olympus BX41, USA). The photomicrographs were taken by means of a digital camera (Nikon Coolpix 885, Japan).

### **Ethical Committee**

The experimental protocol was approved by the Federal University of Ceará Ethics Committee on Animal Research.

## **RESULTS**

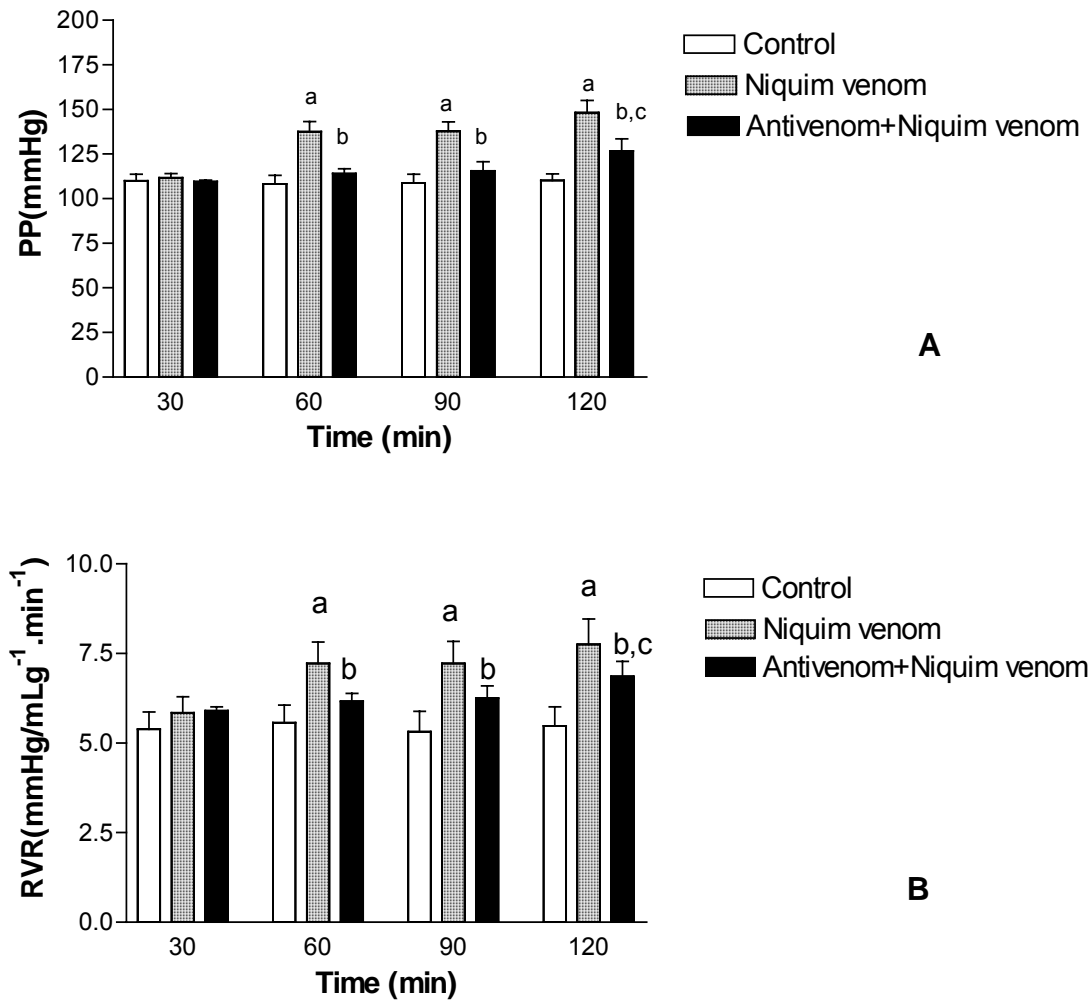
### **Renal Perfusion**

#### **Control group perfused with modified Krebs-Henseleit solution**

The renal parameters of the control group remained stable throughout the experimental perfusion period.

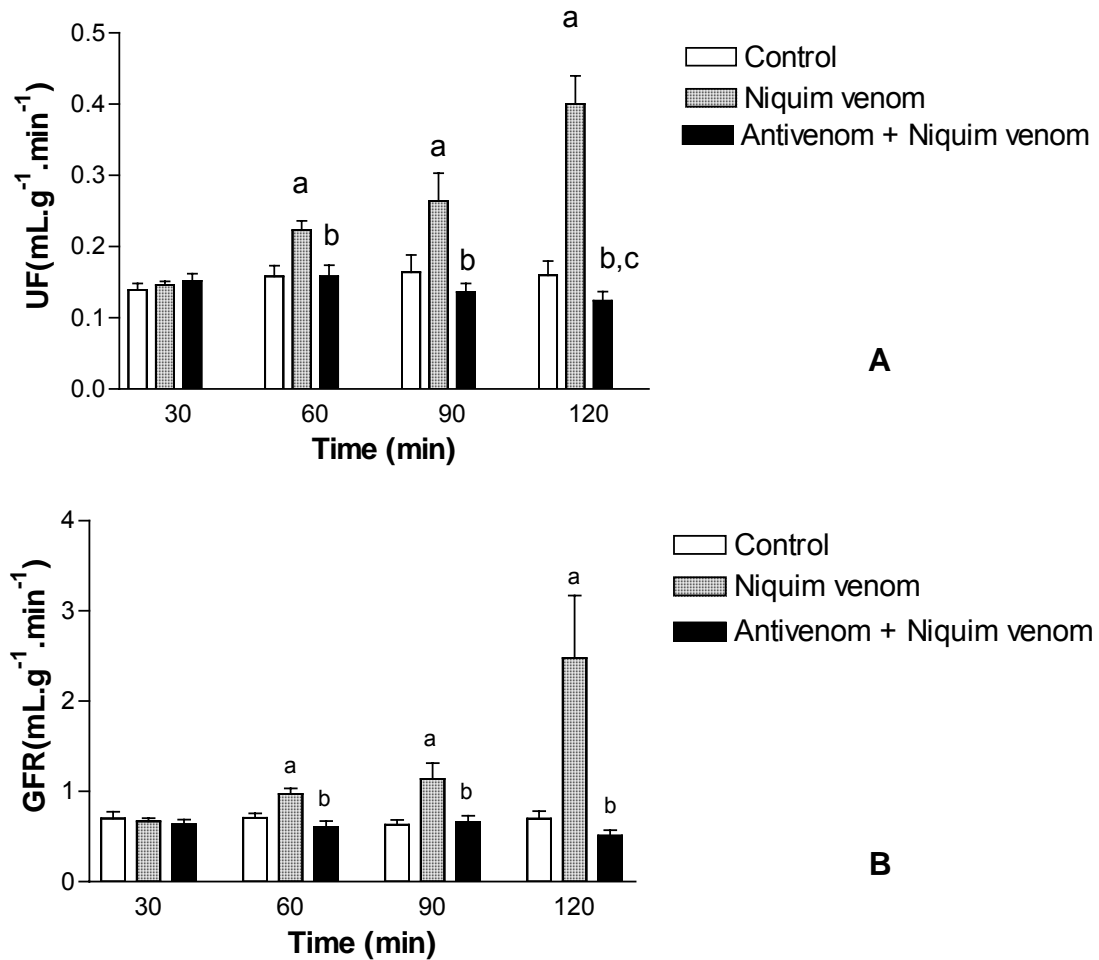
#### **Renal effects induced by *Thalassophryne nattereri* venom**

Previous results showed that *Thalassophryne nattereri* venom can cause alterations of renal function parameters after the 30 minutes of internal control. On the isolated rat kidney *T. nattereri* venom (1 $\mu\text{g}/\text{mL}$ ) increased the perfusion pressure (Figure 1 – A) and renal vascular resistance (Figure 1 – B) at 60, 90 and 120 minutes. UF (Figure 2 – A) and GFR (Figure 2 – B) were also augmented at 60, 90 and 120 minutes when compared with the control group. However, the venom had no effect on the percent of sodium ( $\%TNa^+_{\text{control}} = 81.1 \pm 0.86$ ;  $\%TNa^+_{60} = 78.04 \pm 1.18$ ;  $\%TNa^+_{90} = 76.16 \pm 3.34$ ;  $\%TNa^+_{120} = 79.49 \pm 0.87$ ) and potassium ( $\%TK^+_{\text{control}} = 72.29 \pm 1.12$ ;  $\%TK^+_{60} = 75.41 \pm 0.65$ ;  $\%TK^+_{90} = 71.23 \pm 2.55$ ;  $\%TK^+_{120} = 76.62 \pm 1.04$ ) tubular transport.



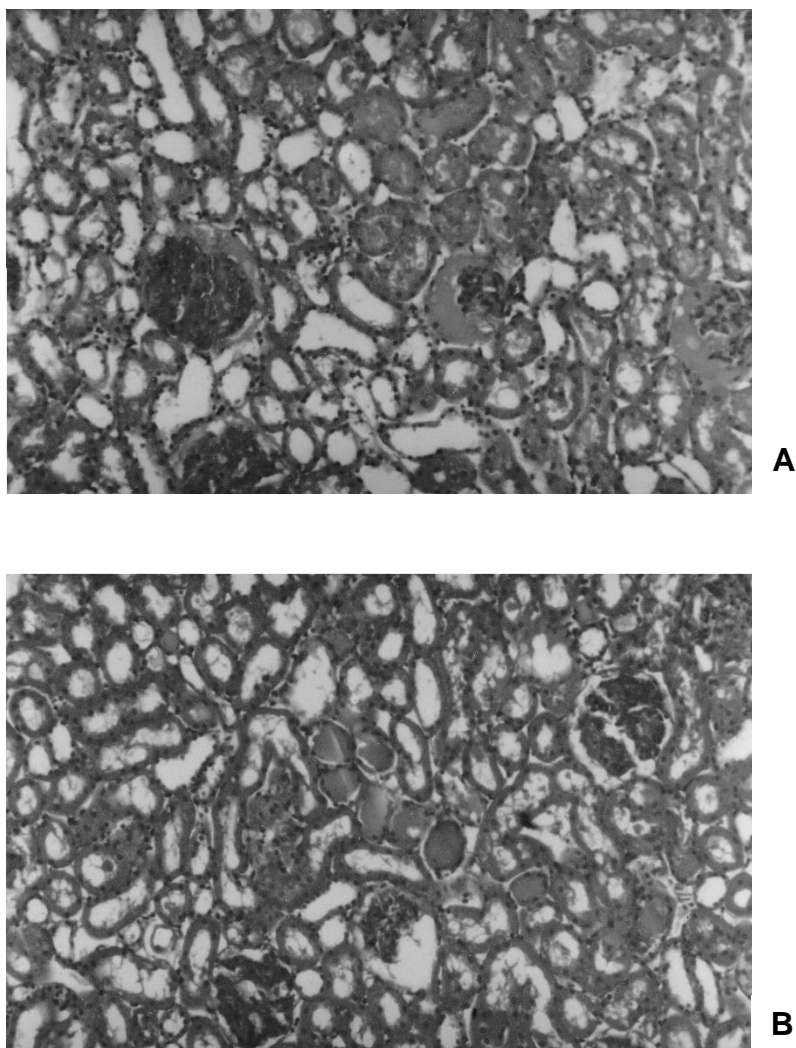
**Figure 1.** Action of the antivenom (1  $\mu\text{g/mL}$ ) on effects of *Thalassophryne nattereri* venom (1  $\mu\text{g/mL}$ ) on perfusion pressure (PP; **A**) and renal vascular resistance (RVR; **B**). Data are expressed as means  $\pm$  SEM from six different animals. ANOVA was employed for statistical analysis by comparison with the control group in which kidneys were perfused with only MKHS with <sup>a,b,c</sup> $p < 0.05$ .

<sup>a</sup>: comparison between control group and niquim venom group; <sup>b</sup>: comparison between niquim venom group and antivenom plus niquim venom group; <sup>c</sup>: comparison between control group and antivenom plus niquim venom group.



**Figure 2.** Action of the antivenom (1 µg/mL) on niquim venom effects (1 µg/mL) on urinary flow (UF; **A**) and glomerular filtration rate (GFR; **B**). Data are expressed as means ± SEM from six different animals. ANOVA was employed for statistical analysis by comparison with the control group in which kidneys were perfused with only MKHS with <sup>a,b,c</sup>p < 0.05.

<sup>a</sup>: comparison between control group and niquim venom group; <sup>b</sup>: comparison between niquim venom group and antivenom plus niquim venom group; <sup>c</sup>: comparison between control group and antivenom plus niquim venom group.



**Figure 3.** Action of the antivenom (1  $\mu\text{g}/\text{mL}$ ) on effects of *Thalassophryne nattereri* venom (1  $\mu\text{g}/\text{mL}$ ). The group that received venom only showed a moderate deposit of proteinaceous material in the tubules and urinary space (A). The kidneys perfused with venom plus antivenom presented a mild deposit of proteinaceous material in glomeruli and tubules (B). Hematoxylin-eosin stain (20x). Canon PowerShot A 95, 5.0 mp.

#### **Effects of *Thalassophryne nattereri* antivenom on isolated kidney**

The administration of antivenom (1 $\mu\text{g}/\text{mL}$ ) caused a slight increase in: PP (PP<sub>control</sub> = 110.40  $\pm$  1.10 mmHg; PP<sub>60</sub> = 115.1  $\pm$  1.60 mmHg; PP<sub>90</sub> = 113.70  $\pm$  4.10 mmHg; PP<sub>120</sub> = 129.00  $\pm$  8.80 mmHg); RVR (RVR<sub>control</sub> = 5.36  $\pm$  0.14 mmHg/mL.g<sup>-1</sup>.min<sup>-1</sup>; RVR<sub>60</sub> = 5.60  $\pm$  0.17 mmHg/mL.g<sup>-1</sup>.min<sup>-1</sup>; RVR<sub>90</sub> = 6.02  $\pm$  0.25 mmHg/mL.g<sup>-1</sup>.min<sup>-1</sup>; RVR<sub>120</sub> = 6.26  $\pm$  0.46 mmHg/mL.g<sup>-1</sup>.min<sup>-1</sup>); in UF (UF<sub>control</sub> = 0.135  $\pm$  0.019 mL.g<sup>-1</sup>.min<sup>-1</sup>; UF<sub>60</sub> =

$0.140 \pm 0.016 \text{ mL.g}^{-1}.\text{min}^{-1}$ ;  $\text{UF}_{90} = 0.159 \pm 0.026 \text{ mL.g}^{-1}.\text{min}^{-1}$ ;  $\text{UF}_{120} = 0.170 \pm 0.047 \text{ mL.g}^{-1}.\text{min}^{-1}$ ); in GFR ( $\text{GFR}_{\text{control}} = 0.778 \pm 0.114 \text{ mL.g}^{-1}.\text{min}^{-1}$ ;  $\text{GFR}_{60} = 0.763 \pm 0.083 \text{ mL.g}^{-1}.\text{min}^{-1}$ ;  $\text{GFR}_{90} = 0.850 \pm 0.382 \text{ mL.g}^{-1}.\text{min}^{-1}$ ;  $\text{GFR}_{120} = 0.709 \pm 0.254 \text{ mL.g}^{-1}.\text{min}^{-1}$ ); in  $\% \text{TNa}^+$  ( $\% \text{TNa}^+_{\text{control}} = 81.1 \pm 0.86$ ;  $\% \text{TNa}^+_{60} = 78.04 \pm 1.18$ ;  $\% \text{TNa}^+_{90} = 76.16 \pm 3.34$ ;  $\% \text{TNa}^+_{120} = 79.49 \pm 0.87$ ) and in  $\% \text{TK}^+$  ( $\% \text{TK}^+_{\text{control}} = 72.29 \pm 1.12$ ;  $\% \text{TK}^+_{60} = 75.41 \pm 0.65$ ;  $\% \text{TK}^+_{90} = 71.23 \pm 2.55$ ;  $\% \text{TK}^+_{120} = 76.62 \pm 1.04$ ) tubular transport. The effects of *Thalassophryne nattereri* antivenom on isolated kidney were not significant when compared with the control group.

### **Antivenom action on renal effects provoked by *Thalassophryne nattereri* venom**

The antivenom was able to revert the renal effects induced by *Thalassophryne nattereri* venom (Figures 1 and 2). Antivenom ( $1 \mu\text{g/mL}$ ) incubated with venom ( $1 \mu\text{g/mL}$ ) inhibited in about 15% the increase of PP and RVR. UF was reduced in 29%, 48% and 69% respectively at 60, 90 and 120 minutes and GFR was diminished in 38%, 42% and 79% at 60, 90 and 120 minutes, respectively.

### **Results of Histological Alterations in Kidney**

Analysis of the control group showed small amount of a proteinaceous material in the renal tubules, although no alteration in the urinary space was detected. The group perfused only with the venom showed a moderate deposit of a proteinaceous material in the tubules and urinary space (8). The group perfused only with the antivenom presented small amount of a proteinaceous material in the renal tubules, similar to the control group. The kidneys perfused with venom plus antivenom presented a mild deposit of proteinaceous material in the urinary space of the glomeruli as well as in the lumina of tubules.



## DISCUSSION

Accidents caused by *Thalassophryne nattereri* represent a medical problem in the northeast of Brazil (1-4). Animal experimentation proved that venom administration can produce nociception, edema, necrosis and lethality (5). Furthermore, venom injection into footpads of mice can provoke local inflammatory lesions, similar to those described in humans (5).

*Thalassophryne nattereri* venom also causes damage in skeletal muscle due to myotoxic effects and vascular alterations followed by impairing regenerative processes (6). We have recently shown that this venom is capable of modifying renal physiology, interfering especially on vascular parameters of isolated rat kidney. These alterations were probably due to myotoxic agents or to a direct effect of the venom on renal cells causing the release of vasoactive factors (8).

It was demonstrated through *in vitro* neutralization assays that antivenom administered to rabbits can completely neutralize the necrotic and nociceptive effects induced by *T. nattereri* venom, therefore justifying the use of serum therapy as an efficient treatment for this type of envenomation (11,18).

As far as we know, the efficiency of *T. nattereri* antivenom in decreasing renal effects induced by *T. nattereri* venom had not been tested. In this study, we observed that *T. nattereri* venom previously incubated with antivenom is able to reduce PP, RVR, UF and GFR. An equal proportion of antivenom is necessary to cause a significant reduction of renal effects provoked by the venom. This is an important finding that shows the efficiency of the antivenom against venom effects on the kidney.

Histological results revealed that the antivenom was able to reduce proteinaceous deposits in the urinary space and renal tubule.

In conclusion, antibodies obtained by a persistent immune response in horses were able to diminish the effects of *T. nattereri* venom on isolated rat kidney. The results also support the possible use of antivenom therapy in treatment of envenomation produced by this fish.

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