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Effects of *Tityus stigmurus* (Thorell 1876) (Scorpiones: Buthidae) venom in isolated perfused rat kidneys

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ABSTRACT

Scorpions belonging to the *Tityus* genus are of medical interest in Brazil. Among them, *Tityus stigmurus* is the main scorpion responsible for stings in the Northeast region. After a sting, the scorpion venom distributes rapidly to the organs, reaching the kidneys quickly. However, there are few studies concerning the renal pathophysiology of scorpion poisoning. In this study, we evaluated the effects of *T. stigmurus* venom (TsV) on renal parameters in isolated rat kidneys. Wistar rats (n = 6), weighing 250-300 g, were perfused with Krebs-Henseleit solution containing 6 g/100 mL bovine serum albumin. TsV at 0.3 and 1.0 µg/mL was tested, and the effects on perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF), glomerular filtration rate (GFR), and electrolyte excretion were analyzed. Effects were observed only at TsV concentration of 1.0 µg/mL, which increased PP ($_{control}PP_{40,}$ = 92.7 ± 1.95; $_{TsV}PP_{40,}$ = 182.0 ± 4.70* mmHg, *p < 0.05), RVR ($_{control}RVR_{40,}$ = 3.28 ± 0.23 mmHg; $_{Tst}RVR_{40,}$ = 6.76 ± 0.45* mmHg, *p < 0.05), UF ($_{control}UF_{50,}$ = 0.16 ± 0.04; $_{Tst}UF_{50,}$ = 0.60 ± 0.10* mL/g/min, *p < 0.05), GFR and electrolyte excretion, with histological changes that indicate renal tubular injury. In conclusion, *T. stigmurus* venom induces a transient increase in PP with tubular injury, both of which lead to an augmented electrolyte excretion.

Key words: 2D-PAGE, kidney, MDCK, venom, *Tityus stigmurus*.

INTRODUCTION

Scorpion stings are the second most frequent cause of poisoning by venomous animals in humans worldwide (Chippaux and Goyffon 2008). In Brazil, scorpion poisoning is a serious public health issue owing to the increase in number of victim notifications, which rose from 12,704 cases in 2000 to 69,053 cases in 2013 (Brasil 2014). The clinical symptoms of scorpion poisoning are due to the presence of neurotoxic peptides in the venom (Possani et al. 1999, Rodriguez De La Vega and

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Possani 2005, Batista et al. 2007), which cause disturbances in the nervous, cardiovascular and muscular systems (Abroug et al. 1995, Zeghal et al. 2000, Mazzei de D'Avila et al. 2002).

Scorpions from the genus *Tityus,* mainly *T. serrulatus*, *T. bahiensis,* and *T. stigmurus,* are responsible for most of the severe poisoning cases in Brazil (Fundação Nacional de Saúde 2001). *T. stigmurus* is the main scorpion responsible for poisoning particularly in the Northeast region, where the species is found (Brasil 2012). Scorpion venoms are composed of mucus, mucopolysacharides, oligopeptides, nucleotides, protease inhibitors, histamine releasers, amino acids, enzymes (hyaluronidases and metalloproteases), and lipids. Several low molecular weight basic proteins (neurotoxins) and bioactive amines (serotonin and histamine) also are present in scorpion venoms, including *Tityus* venom, and act on ion channels in biological membranes (Possani 1984, Gwee et al. 2002, Vasconcelos et al. 2005).

Experimental studies in rats showed hemorrhage and congestion in kidney tissue, causing acute renal failure (Dehghani and Fathi 2012), mainly in young animals, after the administration of *T. serrulatus* venom (Nunan et al. 2003). Moreover, Alves et al. (2005) showed that *T. serrulatus* venom altered renal function parameters by increasing perfusion pressure (PP) and renal vascular resistance (RVR) in the isolated perfused kidney assay.

Although nephrotoxicity is one of the most dangerous and life-threatening effects of animal venoms (Berger et al. 2012, Viswanathan and Prabhu 2011), few studies have shown the renal pathophysiology of scorpionism. Acute renal failure in humans after *Hemiscorpius lepturus* stings has been reported in Pakistan and Iran (Naqvi et al. 1998, Valavi and Ansari 2008). In Brazil, changes in serum levels of creatinine and urea after *T. serrulatus* stings in humans have also been reported, indicating the possible kidney damage caused by the venom toxins (Nunan et al. 2003).

The clinical aspects of *T. stigmurus* stings may vary from mild (with local edema and pain as the main clinical symptoms), moderate (with nausea, vomiting, sweating, salivation, agitation, tachycardia and tachypnea) to severe cases (with profuse vomiting, sweating and salivation, prostration, seizures, pulmonary edema, and shock) (Fundação Nacional de Saúde 2001). However, there is little information about the renal effects of this venom. Thus, this work aimed to evaluate the effects of *T. stigmurus* venom on the renal function of rats, through examination of parameters such as perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF), glomerular filtration rate (GFR), and the electrolyte excretion of sodium, potassium, and chloride. Protein analysis was conducted by gel electrophoresis (2D-PAGE), and the direct effect of the venom in kidney epithelial tubular cells in culture also was evaluated.

MATERIALS AND METHODS

Venom Extraction and Protein Quantification

Tityus stigmurus venom (TsV) was milked from 98 adult scorpions collected in the city of Vitória de Santo Antão (08° 07′ 05″S; 35° 17′ 29″W), 47.2 Km from Recife/PE. Before venom extraction, the scorpions were fasted for one week. Venom extraction was carried out using an electric stimulus of 30 V in the telson to release the venom, which was collected in a capillary tube. The venom was pooled, freeze dried and stored at −20 °C until use.

Protein concentrations were determined using a commercial protein colorimetric assay kit, 2D Quant Kit, according to the manufacturer protocol (GE Healthcare Life Sciences®, PA, USA) with bovine serum albumin (BSA) as a standard of measurement and absorbance at 480 nm.

Protein Analysis by Gel Electrophoresis (2D-PAGE)

Protein samples (400 µg) were dissolved in 250 µL of rehydration buffer (8 M urea, 2% w/v

3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 20 mM dithiothreitol (DTT), 0.5% immobilized pH gradient (IPG) buffer, and 0.002% bromophenol blue) and were loaded onto IPG strips (13 cm with a linear range of IPG pH 3–10) after a brief sonication and centrifugation. Isoelectric focusing was performed in the Ettan IPGphor isoelectric focusing system according to the manufacturer protocol. Before running the two- dimensional electrophoresis, the IPG strips were equilibrated, first for 15 min in fresh buffer (6 M urea, 30% (w/v) glycerol, 2% (w/v) SDS, 100 mM Tris–HCl, pH 8.8) with the addition of 100 mM DTT and subsequently, for 15 min in fresh buffer supplemented with 0.25 M iodoacetamide. The equilibrated IPG strips were transferred onto 12.5% SDS acrylamide gels by use of an Ettan SE 600 Ruby Electrophoresis Unit (GE Healthcare, Piscataway, NJ, USA). The proteins on gels were visualized by staining with Coomassie Brilliant Blue dye (CBBR250) (Candiano et al. 2004). Images of CBB-stained gels were acquired at 300 dpi scanning resolution and 16-bit pixel depth, and then were analyzed with ImageMaster 2D Platinum 7.0 software according to protocols provided by the manufacturer (GE Healthcare Life Sciences®, PA, USA).

Spot Identification by Mass Spectrometry (MS)

In-gel digestion of proteins was performed as described by Shevchenko et al. (2007), with minor modifications. Trypsin was used at a concentration of 25 ng/μL and the reduction and alkylation step was omitted.

Peptides were dissolved in 10 μL 0.1% trifluoroacetic acid (TFA). A saturated solution of alpha-cyano-4-hydroxycinnamic acid (CHCA, 4 mg/mL) in 50% acetonitrile and 0.3% TFA was mixed with an equal amount of sample and spotted on an Anchor Chip 800/384 target plate (Bruker Daltonic GmbH). The sample then was dried in a laminar airflow cabinet for recrystallization. For MS calibration, 0.5 μL of peptide calibration standard (Bruker Daltonik GmbH) was spotted on the target plate with 0.8 μL of CHCA matrix. Samples were analyzed in a matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF)/TOF mass spectrometer (Ultraflex, Bruker Daltonics, Bremen, Germany) in reflectron mode. Peptides with a signal-to-noise ratio above 100 were MS/MS analyzed by using the LIFT technology that is embedded in the Ultraflex MS; on average, ten MS/MS spectra were measured for each protein digest, leading to 2–10 identified peptides. Data were processed using the flex analysis and biotools™ software packages (Bruker Daltonik).

Data analysis was performed using BioTools 3.0 software and MASCOT search engine (Matrix Sciences, UK). Searches were performed using the following parameters: mass tolerance was set to 0.7 Da for fragment ions; trypsin was set as the proteolytic enzyme with one allowed missed cleavage; charge state 1^+ was used; carbamidomethylation of cysteine residues was used as a fixed modification; and oxidation of methionine residues was set as a variable modification.

The NCBInr database was used to identify scorpion proteins either with an in-house or online MASCOT server (Matrix Science, UK). Proteins were both identified and denominated if at least two peptides were identified with a MASCOT peptide ion score higher than 75.

Animals

Male Wistar rats (n = 6), weighing 250 to 300 g, were used in the isolated kidney perfusion assay. The animals were fasted with free access to water 12 h before experimentation. The experimental protocols used in this study were approved previously by the Ethics Committees on Animal Experimentation of the Universidade Federal do Ceará under nº. 539/13.

Isolated Perfused Rat Kidney

For the isolated kidney perfusion assay, the rats were anesthetized with sodium pentobarbital (50

mg/kg, i.p.) and after careful dissection of the right kidney, the right renal artery was cannulated via the mesenteric artery without interrupting the blood flow as described by Bowman (1970). The perfusion fluid consisted of a modified Krebs–Henseleit solution (MKHS) of the following composition (in mmol/L): 114.00 NaCl, 4.96 KCl, 1.24 KH_2PO_4, 0.5 $MgSO_4.7H_2O$, 2.10 $CaCl_2$, and 24.99 $NaHCO_3$. Bovine serum albumin fraction V (BSA, 6g), urea (0.075 g), inulin (0.075 g), and glucose (0.15 g) were added to the solution, resulting in a final perfusion fluid volume of 100 mL. The pH was adjusted to 7.4. In each experiment, 100 mL of MKHS was recirculated for 120 min. The perfusion pressure (PP) was measured at the tip of the stainless steel cannula in the renal artery. Samples of urine and perfusion fluid were collected at 10 min intervals for analysis of the sodium, potassium, and chloride levels by ion-selective electrodes (RAPIDChem 744, Bayer Diagnostic, UK); inulin, as described by Walser et al. (1955) and modified by Fonteles et al. (1983); and osmolality, which was measured in a vapor pressure osmometer (Wescor 5100C, USA). The venom of *T. stigmurus* (TsV) (0.3 and 1.0 µg/mL) was added to the system 30 min after the beginning of each perfusion. The perfusion pressure (PP), renal vascular resistance (RVR), urinary flow (UF), glomerular filtration rate (GFR), the excretion of sodium (ENa^+), potassium (EK^+), and chloride (ECl^-) were determined (Martinez-Maldonado and Opava-Stitzer 1978). The results were compared with those of the internal control group (the first 30 min of perfusion of each animal) and with those of the external control group (one group perfused only with MKHS for 120 min). After each experiment, the kidneys (perfused and non-perfused) were fixed in 10% buffered formalin, processed, and blocked with paraffin, and then sectioned into 5 µm sections. The slides were stained with hematoxylin and eosin (HE) and examined using a light microscope.

Direct Effect on Madin–Darby Canine Kidney (MDCK) Cells

Epithelial MDCK cells were cultivated in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin (10 000 IU/mL), and streptomycin (10 mg/mL). Before each experiment, cells were kept in medium without FBS for 24 h to obtain cells in the G0 phase of cell cycle. Then, cells were removed and incubated with trypsin-EDTA (0.25/0.02% v/v) at 37 °C for about 5 min. After this, the cells were counted in a Neubauer chamber, chamber, suspended in culture medium (1×10^5 cells) and 24 h later used for the experiments.

Cell viability was assessed by the 4,5-dimetilazil-2-il)-2,5 diphenyl tetrazolium (MTT) assay as described by Mosmann (1983). The MDCK cells were plated in 96-well plates at a density of 10^5 cells and treated with different concentrations of TsV (3.12, 6.25, 12.5, 25, 50, 100, and 200 µg/mL). After 24 h of treatment, the cells were incubated with 2.5 mg/mL MTT for 4 h. The formazan crystals that resulted from MTT reduction were dissolved by adding SDS (10%) to each well, followed by incubation for 17 h. The absorbance was read at 570 nm in a microplate reader (Biochrom® Asys Plus), and cell viability was calculated by comparing the resulting absorbances with the mean absorbance of the control wells (without venom, considered to be 100% viable).

Statistical Analysis

Data are expressed as mean ± SEM and were analyzed by ANOVA, followed by the Bonferroni test using GraphPad Prism® 5.0 with significance set at $p < 0.05$.

RESULTS

2D-PAGE and Mass Spectrometry Analysis

The resolution of 400 µg of protein loaded on each gel showed an average of 27 spots with pI ranging between 3 and 10 and molecular masses ranging

between 52 and 17 kDa (Figure 1). The proteins were identified via MALDI TOF/TOF (MS/MS) tandem mass spectrometry, which achieved 14% (4 of 27 spots) successful identifications (Table I). The four identified proteins are isoforms of antarease, a zinc-binding metalloprotease found in venom of *T. serrulatus*.

Renal Effects of TsV

TsV altered all evaluated renal function parameters at a concentration of 1.0 µg/mL, increasing perfusion pressure (Fig. 2a), renal vascular resistance (Fig. 2b), and urinary flow (Fig. 2c), 10 min after the addition of TsV to the system. The glomerular filtration rate increased 20 min after TsV addition (Fig. 2d). The excretion of sodium, potassium and chloride increased significantly at the same concentration (1.0 µg/mL), as shown in Fig. 3, with a consistent increase for the entire duration of experiment. TsV at a lower concentration (0.3 µg/mL) had no effect on the evaluated parameters compared to those of the external control group.

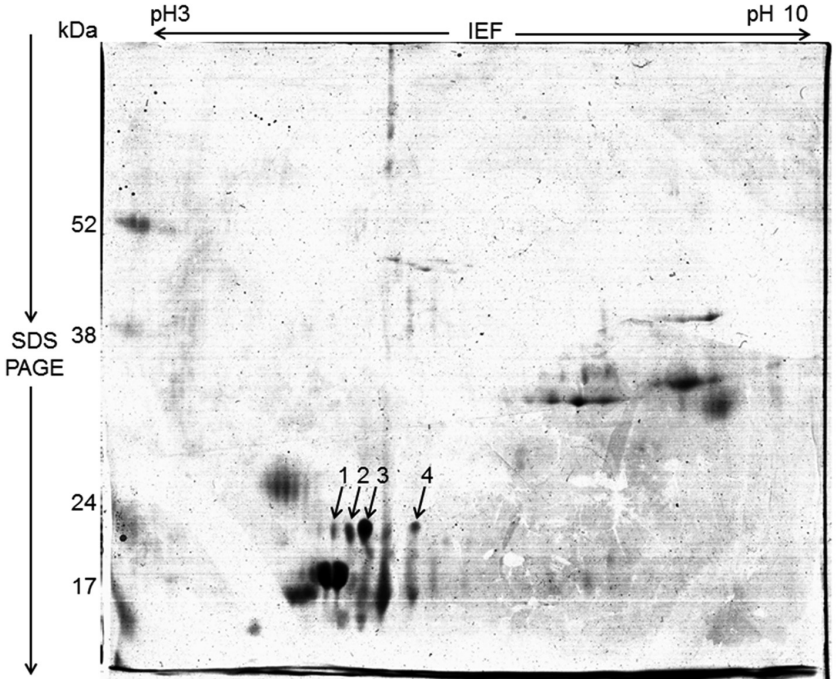

Figure 1 - Proteome profile of *Tityus stigmurus* venom (TsV) on 2D-PAGE.

TABLE I
List of proteins identified in the venom of *Tityus stigmurus* by MALDI TOF/TOF.

Spot[a]	Identity[b]	Organism[c]	Accession[d]	Score[e]	pI[f]	Mr[f]
1	Full=Venom metalloproteinase antarease TserMP_A; Short=VMPA, partial	*Tityus serrulatus*	gi|568818739	77	5.0	25.8
2	Full=Venom metalloproteinase antarease TserMP_A; Short=VMPA, partial	*Tityus serrulatus*	gi|568818740	80	5.0	25.8
3	Full=Venom metalloproteinase antarease TserMP_A; Short=VMPA, partial	*Tityus serrulatus*	gi|568818741	81	5.0	25.8
4	Full=Venom metalloproteinase antarease TserMP_A; Short=VMPA, partial	*Tityus serrulatus*	gi|568818742	78	5.0	25.8

[a]Number allocated according to the numbers used in Figure 1. [b]Identification of proteins annotated by mass spectrometry. [c]Species from which protein was annotated. [d]Access number to the database (nrNCBI). [e]MASCOT score. [f]Theoretical molecular weight and isoelectric point computed from the calculation tool Mr/pI from ExPASy.

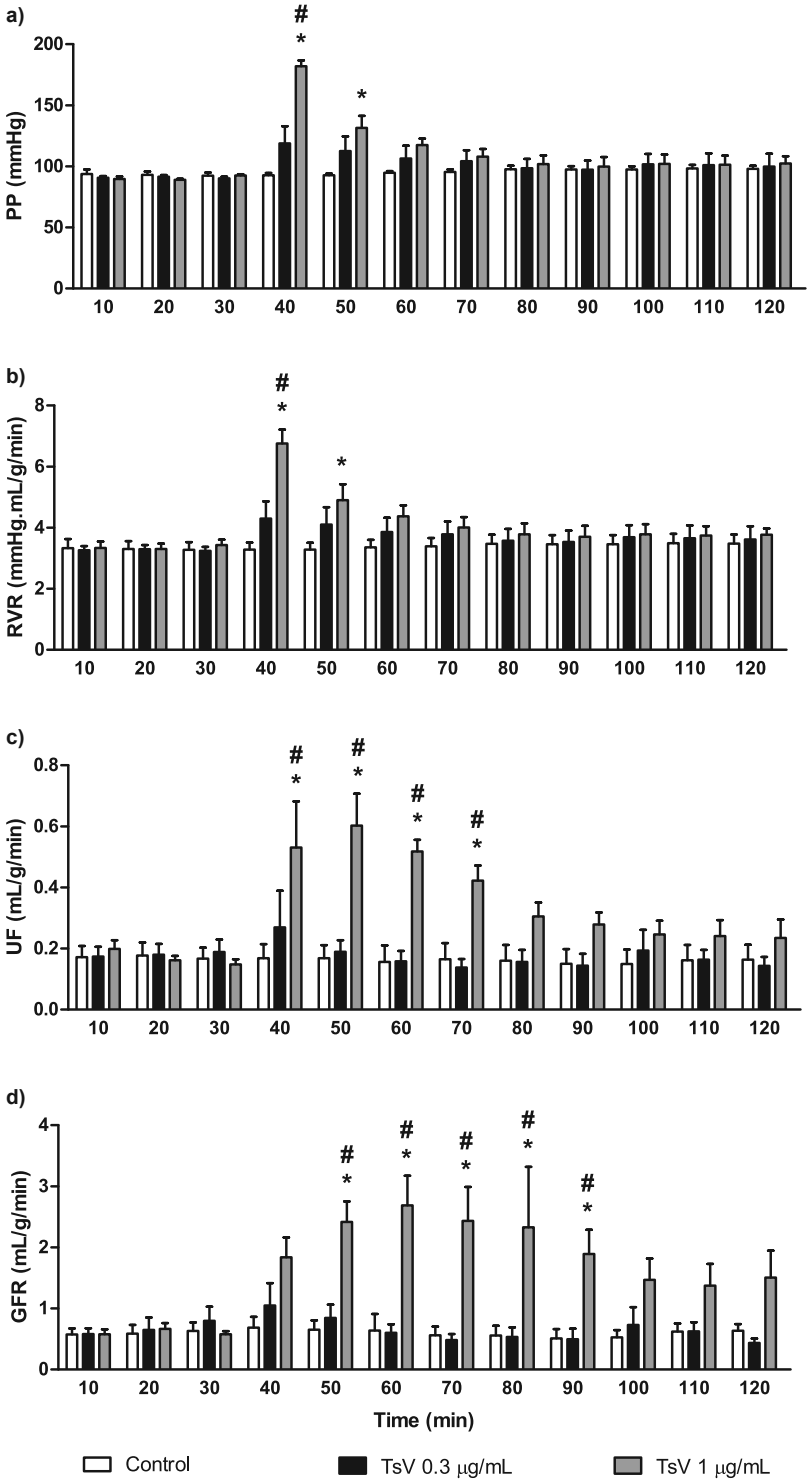

Figure 2 - Effect of *Tityus stigmurus* venom (TsV) on perfusion pressure **(a)**, renal vascular resistance **(b)**, urinary flow **(c)**, and glomerular filtration rate **(d)**. Data are expressed as mean ± SEM from six different animals and were analyzed by ANOVA followed by Bonferroni test. *$p < 0.05$ compared with the corresponding external control group for each interval. $^{\#}p < 0.05$ compared with the group treated with 0.3 μg/mL for each interval.

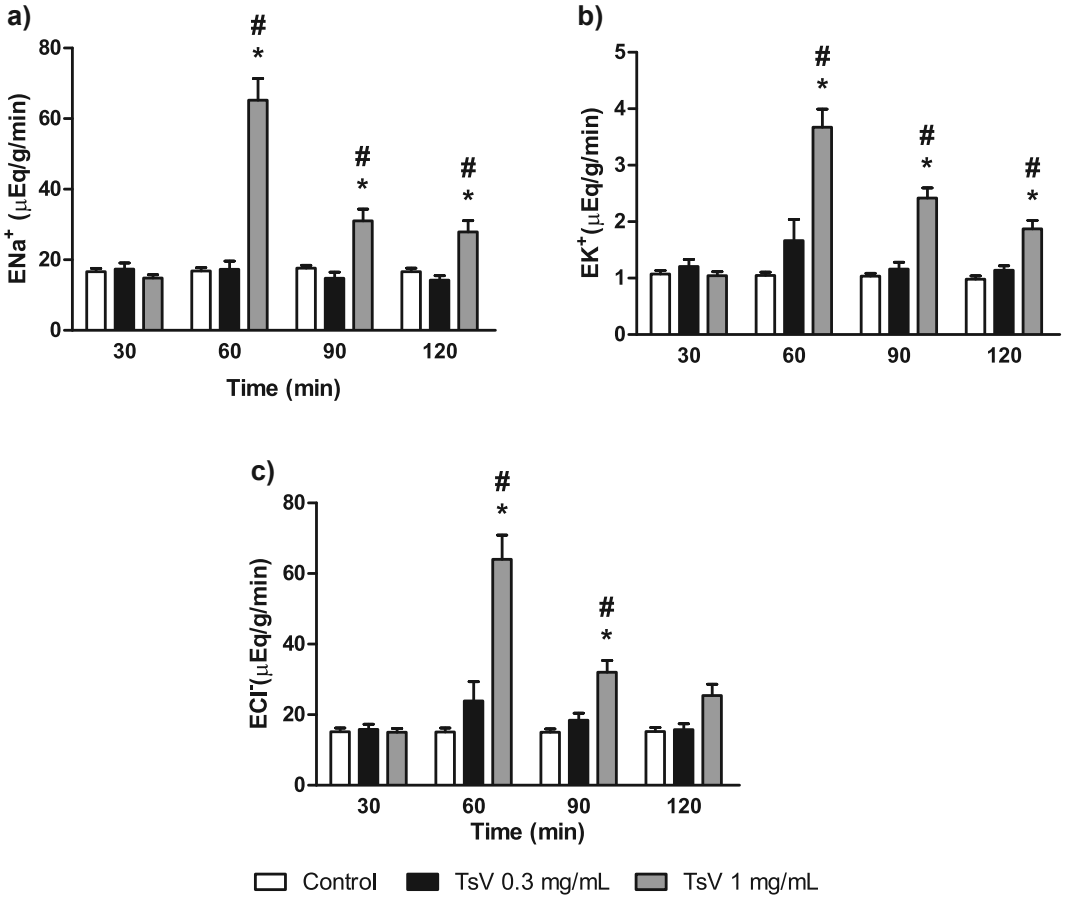

Figure 3 - Effect of *Tityus stigmurus* venom (TsV) on sodium **(a)**, potassium **(b)**, and chloride **(c)** excretion. Data are expressed as mean ± SEM from six different animals and were analyzed by ANOVA followed by Bonferroni test. *$p < 0.05$ compared with the corresponding external control group for each interval. #$p < 0.05$ compared with the group treated with 0.3 µg/mL for each interval.

The histological analysis of the perfused kidneys is shown in Table II. No changes were observed in the glomeruli and tubules of control non-perfused and perfused kidneys, while in both TsV groups, intratubular protein deposits and hydropic degeneration were observed in epithelial tubular cells of the proximal and distal convoluted tubules.

TsV at concentrations up to 200 µg/mL did not show any effect on viability of MDCK cells (Fig. 4).

DISCUSSION

Animal venoms are complex mixtures of toxins, including proteins, peptides, enzymes, and chemicals (Sitprija 2008). Among the components of scorpion venom are enzymes, nucleotide, lipids, biogenic amines, and peptides. Peptides are important components of scorpion venom and have been classified into several families and subfamilies according their structures and biological effects (Barona et al. 2006). 2D-PAGE analysis of TsV identified four spots of antarease-like proteins, which are ubiquitous to a broad range of scorpion species, including in the venom of several *Tityus* spp. Antareases are catalytically active enzymes that penetrate intact tissue and cleave vesicle-associated membrane protein 2 (VAMP2), which is involved in pancreatic secretion. These enzymes may be responsible for scorpionism-induced acute pancreatitis (Ortiza et al. 2014). Almeida et al. (2012) completed a venom gland transcriptome

TABLE II
Qualitative histological analysis of the kidneys perfused with *T. stigmurus* venom (TsV) at 0.3 and 1.0 µg/mL.

Group	Histological changes	Frequency
Control (Kidney not perfused)	No changes were observed in glomeruli and tubules.	-
External control (Kidney perfused with MKHS)	No changes were observed in glomeruli and tubules.	-
TsV 0.3 µg/mL	Normal glomeruli	(6/6)
	Small protein deposits in the PCT and DCT	(4/6)
	Hydropic degeneration in epithelial cells of the PCT and DCT	(1/6)
	Inflammatory cells infiltrate	(0/6)
TsV 1.0 µg/mL	Normal glomeruli	(6/6)
	Small protein deposits in the PCT and DCT	(6/6)
	Hydropic degeneration in epithelial cells of the PCT and DCT	(3/6)
	Inflammatory cells infiltrate	(0/6)

Note: MKHS – modified Krebs–Henseleit solution; PCT – proximal convoluted tubule; DCT – distal convoluted tubule.

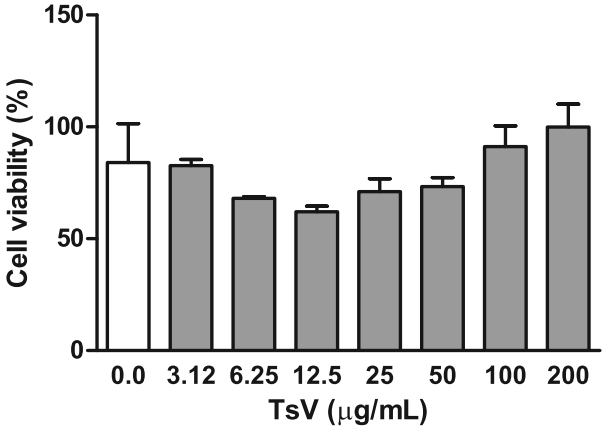

Figure 4 - Effect of *Tityus stigmurus* venom (TsV) at different concentrations on Madin–Darby Canine Kidney (MDCK) cell viability. Data are expressed as mean ± SEM of three independent experiments and were analyzed by ANOVA followed by Dunnett's test.

analysis of *T. stigmurus* and found seven transcripts (clones) represented by six clusters encoding metalloproteases, of which four were similar to antareases-like proteins. Despite this, a previous proteome analysis of *T. stigmurus* venom from Mexico did not find antareases-like proteins (Batista et al. 2007).

Many animal venoms, including venoms from snakes (Morais et al. 2013, Suntravat et al. 2011, Mello et al. 2010), spiders (Kusma et al. 2008), bees (Grisotto et al. 2006), wasps (Vinhote et al. 2011), fishes (Faco et al. 2003), sea anemones (Martins et al. 2009), and scorpions (Alves et al. 2005, Jalali et al. 2011, Heidarpour et al. 2012), can induce renal lesions, mainly acute renal failure. High vascularization, a feature of excretory organs, and the speed at which the scorpion toxins reach the kidneys (at about 15 min after the sting) are the major factors that lead to vulnerability of the kidneys and the development of nephrotoxicity after scorpion stings (Sitprija 2008, Ismail and Elsalam 1988).

T. stigmurus venom increases the RVR and the PP together with urinary flow, which resembles pressure diuresis and could be owing to the presence of biogenic amines in the venom that cause intrarenal vasoconstriction (Badzyńska and Sadowski 2011). Alves et al. (2005) showed that the venom of *T. serrulatus* also increases PP and RVR, probably owing to the direct vasoactive action of the venom, as demonstrated by the mesenteric bed assay. Their results suggest the effect of venom on α1-adrenoceptors. These receptors are found abundantly in the kidney, in both afferent and efferent arterioles (Angelo et al. 2003).

Nevertheless, the quick return of the PP to the basal values could be explained by (1) the direct lesion of the basal membrane of glomerular capillaries due to the elevated PP (Alves et al. 2005) and/or by (2) the presence of pore formation

peptides in the venom, as known for other scorpion species (Sitprija and Sitprija 2012). Taken together, these factors could contribute to the increase in the GFR and UF, as well as to the intratubular protein deposits in the proximal and distal convoluted tubules as observed in the histological analysis.

The hydropic degeneration of epithelial cell of the proximal and distal convoluted tubules implies direct tubular injury. Sitprija (2008) implicated tubular necrosis as the main pathological alteration due to scorpion poisoning. This kind of lesion reinforces the increase in urinary flow and intensifies diuresis, as well as contributes to the decrease in electrolyte reabsorption, with an augmentation of sodium, potassium, and chloride excretion.

The renal tubular transport of electrolytes, especially Na^+ and K^+, usually are affected by animal toxins. Tubular reabsorption of Na^+ is inhibited by most animal toxins as shown in isolated renal perfusion studies, resulting in an augmented Na^+ excretion. The exact site in the renal tubule where inhibition occurs has not been elucidated yet (Sitprija and Sitprija 2012). Scorpion toxins, such as charybdotoxin from *Leiurus quinquestriatus hebraeus* and iberiotoxin from *Hottentota tumulus*, decrease K^+ transport to the tubular lumen (Harvey et al. 1994). However, blocking of calcium-activated K^+ channels (Maxi-K) in the collecting ducts by scorpion toxins can cause clinical hyperkalemia through decreased K^+ secretion in the cortical connecting duct (Sitprija and Sitprija 2012, Ismail et al. 1978).

In addition to the effects on K^+ channels, scorpion toxins in general also affect Na^+ and Cl^- channels, while *T. serrulatus* toxins are well known for opening Ca^{2+} channels (Sitprija and Sitprija 2012), which could contribute to the increase in RVR and PP, and also could participate in the tubular injury due to the deleterious consequences of increased cytosolic Ca^{2+} concentration (Duchen 2000).

In conclusion, *T. stigmurus* venom induces a transient elevation of renal perfusion pressure, with mild glomerular injury and moderate direct tubular lesion, which leads to increased urinary flow, with elevation of sodium, potassium and chloride excretion. No direct cytotoxicity on MDCK cells was observed at concentrations up to 200 μg/mL. Protein analysis by gel electrophoresis (2D-PAGE) revealed the presence of four antarease-like proteins.

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RESUMO

Os escorpiões de interesse médico no Brasil pertencem ao gênero *Tityus*. Dentre eles, *Tityus stigmurus* é o principal responsável por picadas de escorpião na região Nordeste. Após uma picada, a peçonha de escorpião se distribui rapidamente para os órgãos, atingindo os rins depressa. Entretanto, há poucos trabalhos sobre fisiopatologia renal do envenenamento por escorpiões. Neste trabalho, avaliamos os efeitos da peçonha de *T. stigmurus* (TsV) sobre parâmetros renais em rins de ratos isolados. Ratos Wistar (n = 6), pesando 250-300 g, foram perfundidos com solução de Krebs-Henseleit contendo 6 g/100 mL de albumina de soro bovino. TsV nas concentrações de 0,3 e 1,0 μg/mL foi testado e os efeitos sobre a pressão de perfusão (PP), resistência vascular renal (RVR), fluxo urinário (FU), taxa de filtração glomerular (TFG) e excreção de eletrólitos foram analisados. Apenas na concentração de 1,0 μg/mL de TsV foram observados efeitos que aumentaram PP ($_{controle}PP_{40'}$ = 92,7 ± 1,95; $_{TsV}PP_{40'}$ = 182,0 ± 4,70 mmHg*, *p < 0,05), RVR ($_{controle}RVR_{40'}$ = 3,28 ± 0,23 mmHg; $_{Tst}RVR_{40'}$ = 6,76 ± 0,45 mmHg*, *p < 0,05), FU ($_{controle}FU_{50'}$ = 0,16 ± 0,04; $_{Tst}FU_{50'}$ = 0,60 ± 0,10 mL/g/min*, *p < 0,05), a TFG e excreção de eletrólitos, com alterações histológicas que indicam de lesão renal tubular. Em conclusão, peçonha

de *T. stigmurus* induz um aumento transiente da PP com lesão tubular, ambos os quais conduzem a uma excreção elevada de eletrólitos.

Palavras-chave: 2D-PAGE, rim, MDCK, veneno, *Tityus stigmurus*.

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