Nephrotoxicity of Insect and Spider Venoms in Latin America

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Summary: One of the most important and lethal effects of animal venoms is nephrotoxicity. In Latin America, severe acute kidney injury has been reported after accidents with poisonous arthropods such as bees, caterpillars of the genus *Lonomia*, and spiders of the genus *Loxosceles*. In this article the characteristics of these venoms, their probable mechanisms of renal damage, and the clinical picture of the accidents are reviewed.
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Accidents with venomous animals are a significant problem in tropical countries. In Brazil, the number of reported cases has increased (Table 1), whereas lethality has decreased in the accidents caused by snakes, scorpions, and caterpillars (*Lonomia*), probably because of the greater availability and earlier administration of the specific antivenom. The majority of the reported cases are caused by snakes and scorpions. Among the spiders, *Loxosceles* (brown spider) is the most important species for human envenomation. For comparison purposes, in the 2004 annual report of the American Association of Poison Control Centers and Toxic Exposure Surveillance System, 97,263 exposures to animals were reported: 12,424 bee/wasp/hornet; 1,961 caterpillar; 14,950 scorpion; 7,212 snakes, and 25,308 spiders/other insects (*Loxosceles* were 2,859). Only 5 deaths were reported: 3 after snakebite, 1 after bee/wasp/hornet, and 1 after *Loxosceles* accidents.[1]

In this article we review accidents with *Loxosceles*, Africanized bees, and *Lonomia* (caterpillar), which are the arthropods that induce acute kidney injury (AKI) in Latin America (Fig. 1). AKI has not been reported after scorpion accidents, although rhabdomyolysis may occur. The main toxicity of scorpion venom is neuronal, inducing an autonomic storm and myocardial injury, which can cause heart failure and pulmonary edema.[2]

BEE, BROWN SPIDER, AND *LONOMIA* VENOMS

The main characteristics of bee, brown spider, and caterpillar venoms are summarized in Table 2.

Bee venom is composed of a mixture of proteins, peptides, and small molecules. The most important components responsible for the envenomation are phospholipase A_2 and melittin. In addition to these components, bee venom also has hyaluronidase (spreading factor), apamin (a neurotoxin), mast cell degranulating peptide, histamine, dopamine, and noradrenaline, among others.[3-7] Phospholipase A_2, the most active

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Table 1. Cases of Accidents With Venomous Animals and Deaths Reported From 2004 to 2006

	2006		2005		2004	
	Cases	Deaths (%)	Cases	Deaths (%)	Cases	Deaths (%)
Snakes	29,517	79 (0.27)	28,648	113 (0.39)	27,715	114 (0.41)
Spiders	19,306	11 (0.06)	19,537	8 (0.04)	18,138	5 (0.03)
Scorpions	38,734	29 (0.07)	36,012	51 (0.14)	30,313	43 (0.14)
Bees	4,898	14 (0.29)	4,446	13 (0.29)	3,853	9 (0.23)
Lonomia	363	0	347	2 (0.57)	344	3 (0.87)
Other caterpillars	1,866	0	1,934	0	1,432	1 (0.07)
Other animals	2,528	2 (0.08)	2,450	1 (0.04)	2,410	3 (0.12)
Unknown	5,620	2 (0.29)	5,323	4 (0.07)	5,614	4 (0.07)
Total	103,221	137 (0.13)	98,969	192 (0.19)	89,819	182 (0.20)

Data from the Brazilian Ministry of Health.[92]

phospholipase, is abundant in bee venom and is responsible for the degradation of membrane phospholipids, causing the formation of pores and consequent cellular lyses.[6] Hyaluronidase, known as *spreading factor*,[3] induces the degradation of hyaluronic acid and accelerates venom diffusion. Melittin is the most abundant (50%) and the most toxic component of bee venom. Its monomeric form causes cytolysis through the formation of pores in cellular membrane. Melittin acts synergically with phospholipase A_2, exposing the cellular and mitochondrial membrane phospholipids to the action of phospholipase A_2.[3,6,8-10] Melittin acts on erythrocytes, myocytes, hepatocytes, fibroblasts, mast cells, and leukocytes.[3,6,11] Rhabdomyolysis is induced experimentally by either the whole venom or melittin or phospholipase A_2 injection.[12,13] Car-

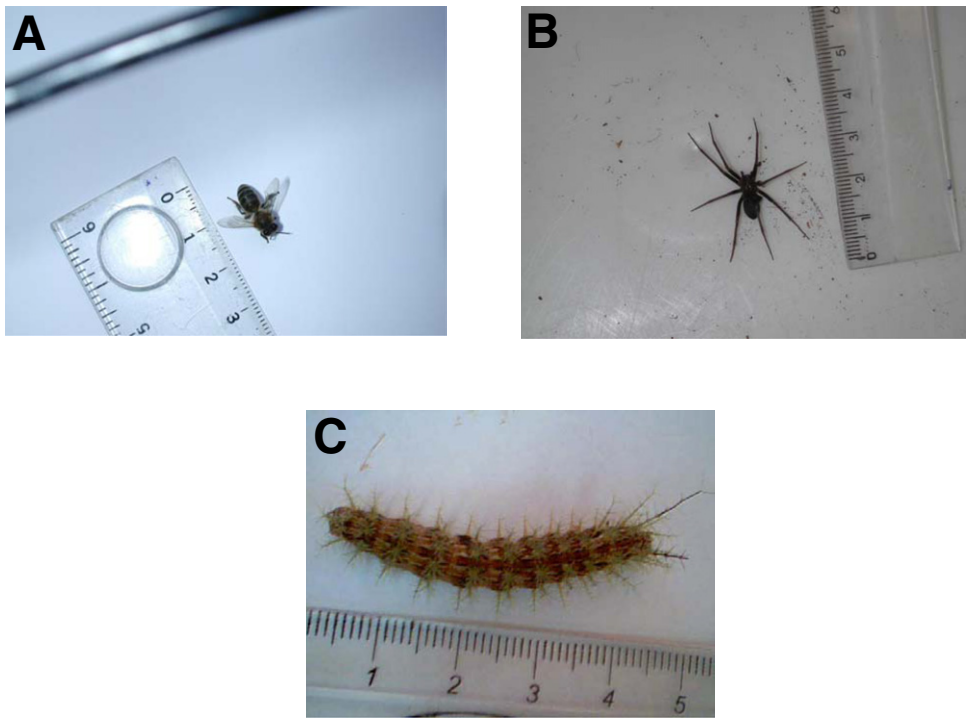

Figure 1. Small but dangerous arthropods responsible for venom-induced acute kidney injury in Latin America. (A) Africanized bee, (B) brown spider (*Loxosceles*), (C) caterpillar (*Lonomia*).

Table 2. Main Components of Bee, Brown Spider, and Caterpillar Venoms

Animal	Component	Molecular Weight	Mechanism	Clinical Effect
Bee *Apis*	Phospholipase A_2	~20,000	Phospholipid hydrolysis, cellular membrane pore formation	Cellular lysis, indirect hemolysis
	Melittin	~12,000	Cellular membrane pore formation, synergic action with phospholipase A_2	Hemolysis, rhabdomyolysis, cardiotoxicity, nephrotoxicity (renal proximal tubule dysfunction)
Brown spider *Loxosceles*	Sphingomyelinase	30,000-35,000	Neutrophil migration, complement activation, cytokine release, platelet aggregation	Inflammation, dermonecrosis, indirect hemolysis and nephrotoxicity
Caterpillar	Lonomin I/V	6,000-18,000	Urokinase-like, factor XIII inactivator	Lysis of preformed thrombi, fibrinogen decrease, fibrinogen degradation products increase
Lonomia achelous	Lonomin III	-	Prothrombin activation independent of phospholipids and calcium ion	
	Lonomin IV	-	Factor Xa-like, prothrombin activation	
	Achelase I Achelase II	22,400-22,700	Plasmin-like	
Lonomia obliqua	Lopap	69,000	Prothrombin activator	Consumptive coagulopathy
	L obliqua Stuart factor activator	43,000	Factor X activator	
	Phospholipase A_2-like	15,000	Indirect hemolytic activity	Intravascular hemolysis
	Hyaluronidases	49,000-53,000	Hyaluronic acid and chondroitin sulfate degradation	Local effects

diotoxicity, increased calcium uptake, and inhibition of sodium and phosphate absorption by proximal tubular cells have been reported with whole venom or melittin administration.[3,14,15]

The mechanism of *Loxosceles* venom action is multifactorial and not fully understood. The clinical picture observed in *Loxosceles* envenoming is owing to the direct action of venom toxins on the cellular membrane, as well as on the extracellular matrix.[16,17] *Loxosceles* venom is a complex mixture of components with toxic and enzymatic activities including alkaline phosphatase, proteases, ribonuclease, lipases, and peptides with insecticide activity.[16-18] The key component responsible for clinical manifestations is sphingomyelinase D (dermonecrotic toxin), which causes neutrophil migration,[17,19,20] complement system activation,[21] cytokine and chemokine release,[22-26] platelet aggregation,[16,27-29] and consequently an intense inflammatory reaction leading to a dermonecrotic lesion. Several components with similar amino acid chains (31-35 kd) were identified and now are known as a family of dermonecrotic toxins with a synergistic action.[30-35] Other important components of *Loxosceles* venom are the metalloproteases. These enzymes degrade components of the extracellular matrix such as fibronectin, entactin, and heparan sulfate,[16,36-38] can act as a spreading factor (astacin),[39,40] and be involved in the development of renal lesions.[41] As in bee venom, hyaluronidase is an important component of this spider toxin and is responsible for the spread of the lesion through the skin by the force of gravity, a characteristic of dermonecrotic lesions caused by *Loxosceles* venom.[38,42-44]

Lonomia venom is present in the caterpillar's bristles, is similar to hemolymph, and is composed mainly of proteins and serine proteases. Although *Lonomia obliqua* and *Lonomia achelous* both cause a hemorrhagic syndrome, they act through different pathways. *L obliqua* venom causes hemorrhage by consumptive coagulopathy resulting from disseminated intravascular coagulation, and *L achelous* venom causes hemorrhage through increasing fibrinolysis.[45] Arocha-Piñango et al[46-48] in Venezuela (where *L achelous* is found), described the mechanism of the envenomation as a primary fibrinolysis. The main components of *L achelous* venom are named *Lonomins*. Lonomins III and IV activate prothrombin either directly (Lonomin III) or through FXa-like activ-

ity (Lonomin IV). Lonomin VIa is a direct factor V activator. Lonomin I/V has a urokinase plasminogen activator-like activity, lyses whole blood clots and fibrin plates, and also causes a dose-dependent degradation of factor XIII/XIIIa. Achelase I and II have a plasminogen-independent direct fibrinolytic activity. The main components of *L obliqua* are *L obliqua* prothrombin activator protease (Lopap), *L obliqua* Stuart factor activator, hyaluronidases, and phospholipase A_2-like toxin. Lopap, whose intravenous injection in rats reproduces the human hemorrhagic syndrome, activates prothrombin in a dose-dependent way, generating thrombin that clots fibrinogen. Its hydrolytic activity is independent of prothrombinase complex components and is increased by calcium. Lopap induces nitric oxide release in endothelial cells, increases the expression of interleukin-8 and cell adhesion molecules intercellular adhesion molecule-1 and E-selectin, and has an antiapoptotic effect. Its activity is inhibited by *Lonomia* antivenom. *L obliqua* Stuart factor activator is a serine-like protease that activates factor X in a dose-dependent way. Two hyaluronidases have been described and named *Lonoglyases*. They degrade hyaluronic acid and chondroitin sulfate, but not heparin or dermatan sulfate. They are important for the local effects of the venom and its passage through the skin. The phospholipase A_2 enzyme is responsible for the hemolytic activity of the venom.[45,49]

Bees

Bees and wasps can cause human injury by 2 mechanisms: allergic reaction, the most common, occurring after a few stings or even one, or direct envenomation when a massive attack with hundreds or thousands of stings occurs. In 1957 swarms of African bees, which have aggressive and migratory behavior and a high reproductive rate, escaped from a research facility in Brazil and hybridized with local European bees forming the so-called *Africanized bees*. Currently, the Africanized bees are found from Northern Argentina to Nevada (United States).[50] Although the composition of the venom is similar, severe attacks are more common with Africanized bees because of their aggressive behavior than with European bees or wasps.

Among the low-molecular-mass components (approximately 25% of the venom composition) are the oligopeptides, amino acids, carbohydrates, and biogenic amines (histamine, serotonin, dopamine, and noradrenalin).[51] Bee venom also has several pheromones that control social activities, including the attraction of other bees to protect the beehive, leading to envenomation by multiple stings.[6]

Clinical manifestations of bee stings can be divided into allergic and toxic reactions. Allergic reactions usually are observed in patients with a history of previous bee stings or asthma or other hypersensitivity disease. These reactions occur immediately after the sting and can lead to death by laryngeal edema.[52] Toxic reactions can be divided into local—pain, erythema, and edema—and systemic, with histamine-like intoxication— urticaria, itching, body burning sensation, nausea, vomiting, abdominal cramps, bronchospasm, respiratory failure, and shock.[53] Rhabdomyolysis and hemolysis can be detected a few hours after the accident. Cardiotoxicity was reported in human beings and in experimental studies.[54-56] In the few cases in which the serum concentration of whole bee venom and its phospholipase A_2 component were measured, the estimated amount of circulating venom was larger and venom urinary excretion persisted longer in the lethal cases.[54] AKI is observed in cases with more than 150 stings.[57] It usually is severe, oliguric, requiring dialysis, concomitant with acute respiratory failure and the need for mechanical ventilation, and has a high mortality rate. Both hypertension and hypotension have been reported with the venom.[54,58-61] Guimarães et al[62] showed an early decrease in blood pressure with rapid recovery after the intravenous injection of the venom in awake rats. Seven to 8 hours later the blood pressure decreased again but returned to normal after 24 hours. Marsh and Whaler[55] reported 2 patterns of arterial blood pressure response after whole bee venom injection in rats. They observed hypotension in the animals with normal baseline blood pressure, whereas in those with mild hypotension blood pressure was increased by the venom. The first response also was observed with the phospholipase A_2 component and the second was observed with

melittin administration. Heme-pigment toxicity, hypotension, and direct nephrotoxicity likely are involved in AKI pathogenesis.[54,63,64] Rhabdomyolysis has been induced experimentally.[12] The intravenous injection of whole bee venom to rats induced an early and significant decrease in glomerular filtration rate (GFR) that persisted after 24 hours. The early GFR decrease was concomitant with marked cortical and medullary renal blood flow decrease, which was not present after 24 hours. Early urinary volume decrease was observed and normalized after 24 hours. In this model neither hypertension nor hypotension nor hemolysis were observed. Rhabdomyolysis was present with massive myoglobin deposition in the lumen of the tubules as well as into the tubular cells. An important finding of this study was that the venom caused direct toxicity in isolated proximal tubule cells.[63] In a consistent way, Han et al[14,15] showed that the addition of whole venom or melittin to cultured renal proximal cells increased lipid peroxide formation, arachidonic acid release, and Ca^{++} uptake, but inhibited Na^{+} and phosphate uptake Na^{+}/glucose cotransporter. In another study, sodium and potassium fractional excretions were increased 3 to 8 hours after intravenous injection of whole venom and the water transport through the collecting ducts was impaired.[65]

Acute tubular necrosis is the histologic finding in human beings, domestic dogs, and in experimental animals after bee envenomation.[54,63-66] Renal function recovery usually occurs by the second week after envenomation.[54,58-61,67]

The treatment of systemic envenomation consists of the administration of antihistaminic drugs, corticoids, and analgesics. The stingers must be removed quickly without concern regarding whether the stings are scraped off or pinched. This quick removal stops venom body inoculation.[68] All types of dialysis, from peritoneal dialysis to hemofiltration, have been used in these patients, but there are no studies specifically addressing this aspect.[54,58,60,61,67]

Brown Spider (*Loxosceles*)

Bites by *Loxosceles* spiders can cause clinical manifestations that are called *cutaneous loxoscelism*, in which only dermonecrotic lesions are present, or *viscerocutaneous loxoscelism*, in which systemic manifestations such as jaundice, hemolysis, rhabdomyolysis, hemorrhage, and/or AKI also are present. Loxoscelism has been reported in South and North America, Asia, Africa, and Australia.[16,17] In Brazil, 3 species of *Loxosceles* are important for human envenomation: *Loxosceles intermedia* (the most frequent), *Loxosceles gaucho*, and *Loxosceles laeta*. The latter also is found in Peru and Chile and is responsible for more severe cases. In fact, *L laeta* is 3 times more lethal for human beings than the other 2 species.[69] In North America *Loxosceles deserta* and *Loxosceles reclusa* are the species of medical importance. Viscerocutaneous loxoscelism has a higher prevalence in areas where *L laeta* is predominant as in Peru (27.2%), Chile (15.7%), and Santa Catarina, Brazil (13.1%). In contrast, in the United States, where *L reclusa* predominates, and in São Paulo, Brazil, where *L gaucho* is the most common species, the frequency of viscerocutaneous loxoscelism is lower (0.7%-1.8% and 4.2%, respectively).[17] Viscerocutaneous loxoscelism was diagnosed in 13.1% of 267 loxoscelism cases reported in one area of Brazil where *L laeta* is the main species. The investigators reported jaundice in 69%, oliguria in 46%, hemorrhage in 26%, and shock in 3% of the patients. AKI occurred in 6.4% of the patients, and most of them were diagnosed more than 24 hours after the bite. Four patients died (1.5%), all of them younger than 14 years old.[70] In contrast, a retrospective study of 359 cases of loxoscelism in another area of Brazil where *L gaucho* is the most important species reported only 13 cases (3.6%) of the viscerocutaneous form, and none of the patients developed AKI or died.[71]

The factors likely associated with AKI development are hemolysis, rhabdomyolysis, hypotension/shock, and direct venom nephrotoxicity.[72] Pigment-induced acute tubular necrosis was reported in human necropsies of viscerocutaneous loxoscelism.[73] The proposed mechanism for hemolysis is that the sphingomyelinase activates an endogenous metalloproteinase that cleaves glycophorins, making the erythrocytes susceptible to lysis by complement.[21] Many en-

zymes, such as hyaluronidase, are responsible for the spread of the venom with gravity increasing and deepening the tissue lesions, amplifying the inflammatory response, local edema, and ischemia, leading to rhabdomyolysis. Although the direct myotoxic effect, evaluated by creatine kinase serum levels, has not been observed experimentally with *L gaucho, L laeta*, and *L intermedia* venoms,[74] local myonecrosis was observed 24 hours after the intradermic injection of *L intermedia* venom in rabbits.[75] Hemoglobin and myoglobin are well-known nephrotoxic agents mainly in the presence of hypotension or dehydration, frequently found in viscerocutaneous loxoscelism. However, even in the absence of hypotension or hemolysis, the intravenous injection of *L gaucho* venom in rats induced AKI and rhabdomyolysis. AKI was characterized by an early and important decrease in urinary volume (9.4 ± 0.3 versus 5.2 ± 0.6 μL/min, $P < .01$), GFR (0.92 ± 0.06 versus 0.30 ± 0.04 mL/min/100 g, $P < .01$), and renal blood flow (4.6 ± 0.3 versus 1.9 ± 0.2 mL/min, $P < .01$). Acute tubular necrosis associated with the presence of myoglobin also was observed.[76]

The venom mechanisms causing tubular cell toxicity are not completely understood. Experimental administration of *L intermedia* venom or its purified recombinant dermonecrotic toxin to rats induced AKI and acute tubular necrosis associated with the presence of the venom/toxin in the renal tissue.[41,77] The addition of the toxin to MDCK cell culture (in the presence of serum) induced cytotoxicity.[77] On the other hand, the addition of the *L gaucho* venom to fresh isolated proximal tubules of rats (without any serum component) did not cause cytotoxicity.[76] These controversial data might be related to specific serum component-mediated cytotoxicity or to differences among species.

In the mild cutaneous cases treatment is only symptomatic. Some investigators recommend the use of dapsone and colchicine in more severe cases to inhibit polymorphonuclear cell degranulation and to reduce local inflammation. However, we must take into consideration that dapsone has many adverse effects such as hepatitis, leukopenia, methemoglobinemia, and hemolytic anemia. When the antivenom is available, as in Brazil, its administration is recommended in patients with large cutaneous or necrotic lesions and in the viscerocutaneous form.[17] In the viscerocutaneous form, vigorous hydration and urinary alkalinization should be established early to avoid pigment-induced AKI.

Caterpillar (*Lonomia*)

Lonomia is a moth and provokes accidents only in its caterpillar phase when it has venomous bristles. Usually the accidents occur in rural areas and can be considered an occupational hazard.[78-81] The accidents are more numerous during the spring and summer when the moth is in its caterpillar phase and people wear clothes that leave larger body areas exposed to bristle contact.[82]

Accidents caused by caterpillars of the species *L obliqua* have been observed with increasing frequency in recent years in Brazil. The first Brazilian case of severe bleeding related to contact with a Lepidoptera caterpillar was described by Alvarenga.[83] In the late 1980s and early 1990s there was a considerable increase in the number of hemorrhagic accidents in rural areas of southern Brazil.[81,84-86] In the State of Santa Catarina, the number of cases increased from around 30 per year in 1989 to 1995 to more than 200 per year in 1996 to 1999, decreasing to around 100 cases per year in 2000 to 2003.[81] The cause of this major increase in the number of cases is not clear, but probably is related to deforestation and the progressive reduction of natural predators.[87]

Patients experience severe burning pain at the site of contact (generally the upper limbs), nonspecific symptoms, and bleeding.[48,81,83,88] Hemorrhagic syndrome is one of the most striking features of *Lonomia* envenomation. It can manifest as extensive ecchymosis in the contact areas, hematomas, gum bleeding, scar bleeding, epistaxis, hematemesis, melena, hematuria, and metrorrhagia.[46-48,80,81,85,88] Intracranial and pulmonary hemorrhage and bleeding in unusual sites, such as the spinal cord, thyroid, and intraperitoneal cavity, may occur in the most severe cases.[81,84,85,89] Zannin et al, studying 105 accidents with *Lonomia obliqua*, observed that coagulation changes occurred early and

were characterized as consumptive coagulopathy, without thrombocytopenia.[80] Remarkably, severe cases have not been reported any longer after the introduction of *Lonomia* antivenom.[81,84,85,88,89]

AKI is found in up to 5% of patients and microscopic or gross hematuria are frequent concomitant manifestations.[84,85,89-91] In a recent study, 2,067 cases seen in the State of Santa Catarina, Brazil, from 1989 to 2003 were evaluated.[81] A total of 39 AKI cases (defined as a plasma creatinine level of ≥1.5 mg/dL) were observed (1.9%). In 1995 *Lonomia* antivenom was introduced in the treatment of severe accidents. Before 1995 there were 175 reported cases, 4 with AKI (2.9%) and 7 deaths. From 1995 to 2003 there were 1892 cases reported, 34 with AKI (11 needed dialysis) and no deaths.[81] To evaluate the factors associated with renal injury, the AKI cases were compared with 34 control cases without AKI paired by age, sex, and time to treatment. AKI patients had accidents more frequently with caterpillar colonies, caterpillar contact was mainly on the hands, and nonspecific and bleeding symptoms were more frequent. Laboratory tests in AKI cases disclosed significantly more altered hematologic parameters when compared with control patients. Clotting time was more than 30 minutes in 82% of the AKI cases versus 0% in controls, they had a lower number of platelets (137 ± 80 versus 203 ± 76 $10^3/mm^3$), and lower hemoglobin levels (12.1 ± 3.0 versus 13.7 ± 1.9 g/dL).[79] There are 2 *Lonomia*-induced AKI case reports describing renal biopsies in the literature. One was a previously hypertensive 67-year-old woman who presented with anuria soon after contact with a caterpillar colony. She required dialysis for 26 days. Renal biopsy was performed on the 17th day and showed 8 glomeruli with normal appearance, despite thickening of the Bowman's capsule and focal tubular atrophy.[90] The other case was a 37-year-old pregnant woman who had anuria and gum bleeding 5 to 6 hours after the accident. Twenty-four hours after the accident the patient developed genital hemorrhage, with a diagnosis of abruptio placentae. Delivery was induced and she gave birth to a live child. She developed hemorrhagic shock after the delivery, oliguria, and AKI. The oliguria remained for 2 weeks and hemodialysis was required for 3 weeks. Renal biopsy was performed on day 27 and showed regenerating acute tubular necrosis.[91] To date, there is no experimental model available for the study of the mechanisms causing AKI. Hemodynamic changes secondary to bleeding or direct nephrotoxicity are considered factors possibly causing renal injury. Cortical necrosis resulting from intravascular coagulation also is possible because 4 patients with AKI never recovered renal function.[81]

Adequate support therapy and administration of *Lonomia* antivenom may improve the accident outcome. In fact, in the State of Santa Catarina, Brazil, no death has been reported after the introduction of the antivenom.[81]

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