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Cardiotoxicity Potential of *Loxosceles intermedia* Venom in *Cavia porcellus*

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ABSTRACT

***Background*:** Bites from spiders of the genus *Loxosceles*, popularly known as the brown recluse spider, can cause a syndrome called loxoscelism, which is classified into cutaneous and viscerocutaneous forms. The cutaneous form, which is more common, causes the development of a necrotizing lesion with gravitational spread at the bite site. The viscerocutaneous form, which is rarer, includes systemic symptoms such as intravascular hemolysis, thrombocytopenia, and acute kidney injury, which can lead to death. However, clinical reports suggest a potential cardiotoxic role of *Loxosceles* venom, although this has been little studied. It should be noted that phospholipase-D is the main toxin present in the venom and has been implicated as the factor responsible for the development of cardiac dysfunction. Given this context, the present study sought to deepen the understanding of the effects of *L. intermedia* spider venom on the hearts of guinea pigs, using a detailed approach involving cardiac biomarkers, electrocardiograms, and histopathological analysis through optical and electron microscopy.

***Materials, Methods & Results*:** Eighteen male guinea pigs (*Cavia porcellus*) weighing 600 g were used. Of these, 16 animals received intradermal injections of *Loxosceles intermedia* venom at doses ranging from 11.6 to 350 µg diluted in 0.5 mL of 0.9% saline, and 2 animals received only 0.5 mL of 0.9% saline, serving as controls. Cardiac function was assessed by serial electrocardiograms and by measuring serum cardiac biomarkers, such as creatine kinase (CK) and its MB fraction (CK-MB), lactate dehydrogenase (LDH), troponin I (TnI), N-terminal fragment of B-type natriuretic peptide (NT-ProBNP) and D-dimer at 24 and 72 h post-injection. After 72 h, the animals were euthanized for histopathological and ultrastructural analysis of cardiac tissue using optical and transmission electron microscopy. Survival time varied inversely with the venom dose. Electrocardiograms recorded before envenomation showed no abnormalities. However, after *Loxosceles* envenomation, ECG changes were observed, including cardiac arrhythmias such as junctional beats and supraventricular extrasystoles, paired ventricular extrasystoles associated with isolated junctional beats, isolated junctional beats and atrioventricular block, junctional arrhythmia, and isolated atrial extrasystole. Elevated CK, CK-MB, LDH, and TnI levels were observed. Histopathological changes included diffuse cardiac congestion and focal hemorrhage, while ultrastructural analysis revealed autophagic vacuoles and mitochondrial damage in cardiac fibers.

***Discussion*:** This research was the 1st to conduct a clinical study on systemic loxoscelism and its cardiovascular impacts. The data obtained demonstrate that loxoscelism envenomation can cause significant cardiac and biochemical alterations, with rapidly manifesting effects, such as atrial and ventricular premature beats. Atrial premature beats result from early atrial ectopic beats, and ventricular premature beats present as beats originating early in the ventricle, with a post-extrasystolic pause. In envenomations, ventricular premature beats are associated with damage to cardiac muscle fibers due to myotoxic activity and impaired perfusion of cardiac tissue. Junctional beats, which are early ectopic beats originating in the atrioventricular junction, were also observed. Therefore, these results indicate that loxoscelism venom can directly interfere with cardiac electrophysiology, causing conduction disturbances and arrhythmias. Furthermore, the venom was capable of generating histological lesions consistent with myocardial damage that may also indirectly contribute to impaired cardiac function through mechanisms such as hypoxia and hemorrhages.

Keywords: *Loxosceles intermedia*, loxoscelism, guinea pig, cardiotoxicity, cardiac biomarkers, electrocardiogram.

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INTRODUCTION

Accidents caused by spiders of the genus *Loxosceles*, known as brown recluse spiders, account for a significant portion of envenomation cases involving venomous animals worldwide [8]. This is due to the high toxicity of *Loxosceles* venom, which, although produced in small volumes (2 µL), contains between 20 and 200 µg of total protein and has a complex biochemical composition capable of inducing deleterious effects in mammals [19-22].

The heart has also been described as the organ with the 2nd highest concentration of *Loxosceles* venom [5]. The cardiotoxic potential of loxoscelism was observed in rats exposed to the venom, which showed increased serum levels of creatine kinase and the MB fraction, as well as reduced systolic blood pressure and heart rate [7]. Despite the scarcity of specific studies on the cardiotoxicity of *Loxosceles* venom, there are clinical reports that indicate its cardiotoxicity. Kounis syndrome has been described in a 9-year-old child bitten by *L. laeta*, with ST-segment elevation [15]. Another case involved a 16-year-old adolescent who developed myocarditis, disseminated intravascular coagulation, and severe hemolysis after an accident with *L. reclusa* [12]. Furthermore, a 43-year-old adult, after an accident with *L. rufescens*, developed toxic myocarditis with significant systolic dysfunction, dilated left ventricle, and reduced ejection fraction confirmed by echocardiography and magnetic resonance imaging, in addition to elevated troponin and NT-pro-BNP levels [6]. Therefore, this study aimed to investigate the cardiotoxic effects of *L. intermedia* venom in guinea pigs.

MATERIALS AND METHODS

Animals

A total of 18 male guinea pigs of the English Short Ear strain (*Cavia porcellus*), with an average weight of 600 g, were used in this experiment. The animals were kept in the vivarium of the Multidisciplinary Animal Research Unit of the Veterinary School of the Federal University of Minas Gerais (UFMG), Brazil, where they were housed in individual metal cages, with food and water available *ad libitum*, under controlled environmental conditions.

Venom

A pool of *L. intermedia* venom from the Venom Bank of the UFMG Immunology and Biochemistry Laboratory was used. The protein concentration of the venom was determined using the Lowry method, with the establishment of a standard curve, where an average calibration factor was obtained, resulting in an average concentration of 3.5 µg/µL.

Experimental protocol

Sixteen animals were challenged with *L. intermedia* venom intradermally, using a sterile disposable insulin syringe (0.5 mL Ag 0.30 X 8 mm)[1]. The injections were administered in the interscapular region, previously trichotomized. The doses administered ranged from 11.627 µg to 350 µg (Table 1), with 0.9% saline solution[2] added to a final volume of 0.25 mL, to standardize the volume applied. These doses were chosen based on the protein concentration of the venom that an adult spider injects during a bite, approximately 20-200 µg of total protein [3,20-22], as well as the LD50 value in mice [1]. As a control, 2 animals received 0.25 mL of 0.9% saline solution under the same conditions.

Table 1. Concentrations of *Loxosceles intermedia* venom injected intradermally into guinea pigs (*Cavia porcellus*).

Venom concentration (µg)	
0 µg (control)	-
0 µg (control)	-
11.627 µg	Low concentration
12.789 µg	Low concentration
14.068 µg	Low concentration
15.475 µg	Low concentration
17.795 µg	Low concentration
18.725 µg	Low concentration
21.533 µg	Regular concentration
24.762 µg	Regular concentration
28.477 µg	Regular concentration
29.995 µg	Regular concentration
47.985 µg	Regular concentration
76.775 µg	High concentration
105 µg	High concentration
140 µg	High concentration
210 µg	High concentration
350 µg	High concentration

Electrocardiogram (ECG)

The animals underwent cardiac assessment using a computerized electrocardiogram (INCardio Agile®)[3]. The test was carried out in the pre-cordial leads (I, II, III, aVL, aVF, aVR) and analyzed in lead II (DII), at a speed of 50 mm/s and amplitude of 2N, for 5 min [4]. Electrocardiograms were recorded at 5 times: time zero (T0), immediately after the application of *L. intermedia* venom/saline solution, and 12, 24, 48 and 72 h after the challenge.

Cardiovascular biomarkers

Blood samples were taken by puncturing the jugular vein at previously established times: 24 and 72 h after the application of *L. intermedia* venom. The collected blood was stored in tubes containing clot activator gel[4] and 3.2% sodium citrate anticoagulant4 to obtain serum and plasma, respectively. The samples were centrifuged at 178.36 *g* for 10 min for serum and, 131.04 *g* for 15 min for plasma. To quantify cardiac biomarkers, serum levels of creatine kinase (CK), creatine kinase MB (CK-MB) and lactate dehydrogenase (LDH) were measured using commercial kits (Biotécnica®)[5] using automatic biochemical analyzers, using the spectrophotometry method (Smart 200 Plus)[6]. For troponin I, the N-terminal fragment of the B-type natriuretic peptide (NT-ProBNP) and D-dimer, ECO Diagnóstica® kits[7] were used for analysis using the fluorescence immunoassay technique with a europium marker in ECO Reader F200 equipment[7].

Anatomical-histopathological evaluation

The animals that did not die spontaneously within 72 h of the application of *L. intermedia* venom were euthanized in accordance with the regulations of the National Council for the Control of Animal Experimentation (CONCEA) and the guidelines of the Ethics Committee on the Use of Animals (CEUA). Euthanasia was carried out using the inhaled anesthetic isoflurane[8]. During necropsy, heart fragments were obtained, fixed in 10% buffered formalin[9], embedded in paraffin[9], cut in a micro tome (4 µm-thick sections), and stained with hematoxylin-eosin (H&E)[10] method, and analyzed by light microscopy[11]. Other samples of 0.5 cm of heart were immediately fixed in glutaraldehyde[10] for subsequent ultrastructural analysis by transmission electron microscopy (Tecnai G2-12 Spirit Biotwin FEI-120 kV®)[12].

Statistical analysis

Statistical analysis was carried out using GraphPad Prism software. The correlation between the variables was analyzed using Pearson or Spearman coefficients, depending on the distribution of the data. Scatter plots, correlation coefficients (r) and *P*-values were generated, with $P < 0.05$ considered statistically significant. Spearman's coefficient was used for non-parametric data or when the relationship between the variables was not linear. The interpretation of the results considered both the strength and significance of the correlations.

RESULTS

Survival rate

All animals from the lower concentration (11.627 to 18.725 µg) died from euthanasia, 72 h after the inoculation. Animals from the regular concentrations (21.533 to 47.985 µg) died around 24 h after inoculation, whilst animals from the higher concentrations (76.775 to 350 µg) died approximately 30 h after inoculation (Figure 1).

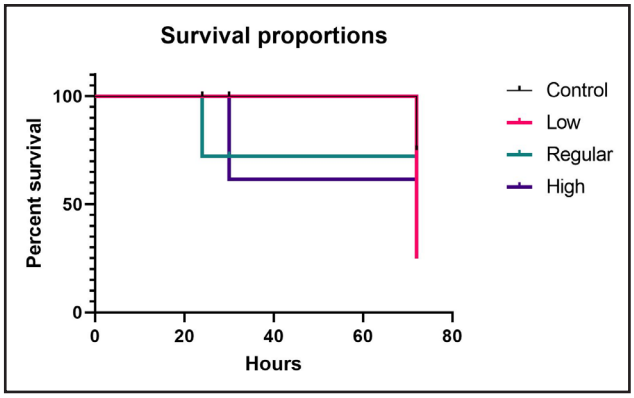

Figure 1. Kaplan-Meyer curve estimation of the survival curve of *Cavia porcellus* that received saline (control group) and progressive doses of *Loxosceles intermedia* venom: low, regular, and high doses.

Electrocardiogram

The ECG recordings made prior to poisoning showed no abnormalities. All the guinea pigs had a regular sinus rhythm (RRS) [Figure 2], considered a physiological heart rhythm, with the occurrence of a P wave preceded by a QRS complex [4]. After, *Loxosceles* envenomation, only 1 animal inoculated with an average dose of 28.477 µg of venom showed no changes in the electrocardiographic tracing.

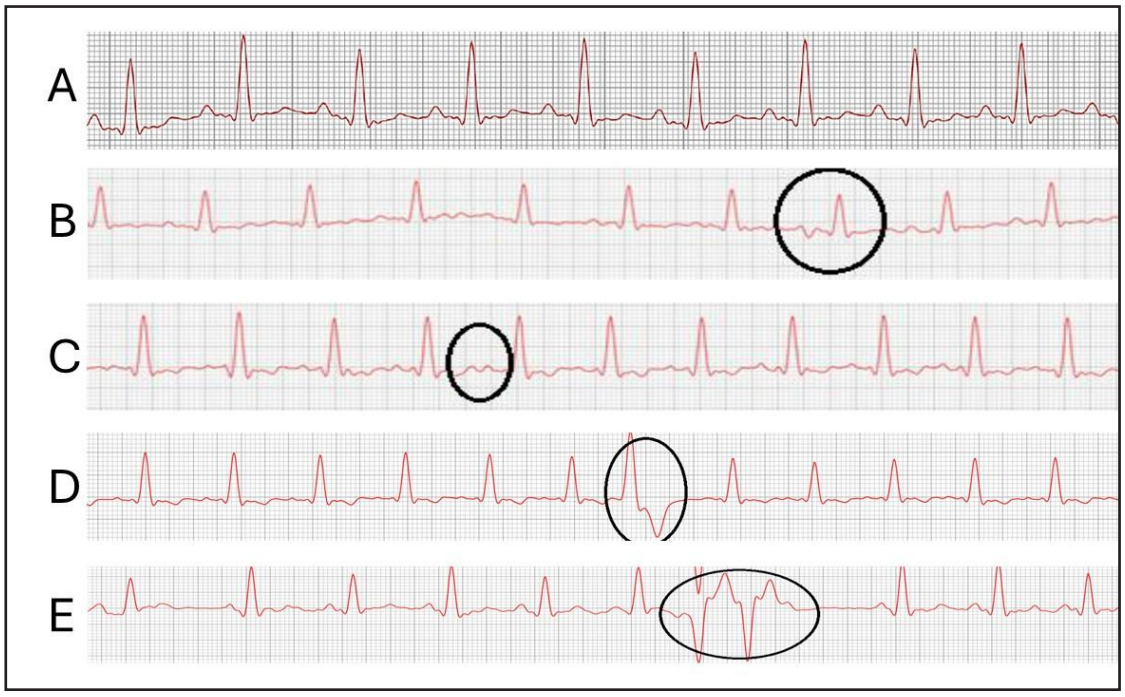

Figure 2. Electrocardiographic tracings showing sinus rhythm and cardiac changes in *Cavia porcellus* after intradermal application of *Loxosceles intermedia* venom. A- Sinus rhythm at time zero (D2 derivation, 50 mm/s, N). B- Sinus rhythm after venom application, with isolated junctional beat circled in black (D2 derivation, 50 mm/s, N). C- Sinus rhythm after venom application, with isolated atrial extrasystole circled in black (Lead D2, 50 mm/s, N). D- Sinus rhythm after venom application, with isolated ventricular extrasystole circled in black, probably originating in the right ventricle (Lead D2, 50 mm/s, N). E- Sinus rhythm after venom application, with paired ventricular extrasystoles circled in black, probably originating in the left ventricle (Lead D2, 50 mm/s, N).

In the 1st h after intradermal application of *L. intermedia* venom, ECG changes were observed, including cardiac arrhythmias such as junctional beats and supraventricular extrasystoles at dose of 12.789 µg. Isolated supraventricular extrasystoles occurred at doses of 15.475 µg, 18.725 µg, 21.533 µg, 24.762 µg and 28.477 µg. After 12 h, supraventricular extrasystoles were still observed (at doses of 18.725 and 21.533 µg). At doses of 24.762 µg and 28.477 µg, there were paired ventricular extrasystoles associated with isolated junctional beats.

Twenty-four h after *Loxosceles* venom administration, isolated junctional beats and atrioventricular block (11.627 µg dose), junctional arrhythmia (12.789 µg dose), and isolated atrial extrasystoles (47.985 µg dose) were observed. After 48 h, the previously observed changes were maintained. However, ventricular pre-excitation (15.475 µg dose) was observed, in addition to isolated atrial extrasystoles and episodes of focal atrial tachycardia (18.725 µg dose). After 72 h, isolated atrial extrasystoles continued to be observed, but only at the 11.627 µg dose.

Cardiovascular biomarkers

Blood samples from the animals that were subjected to the higher doses (105 to 350 µg) of venom were insufficient for cardiovascular biomarkers' analysis, and therefore only control, low, regular doses and 76.775 µg are described forward for these analysis. Of the animals challenged with *L. intermedia* venom, 5 showed CK levels above the maximum normal limit [14], 24 h after envenomation, at doses of 12.789 µg; 15.475 µg; 18.725 µg; 24.765 µg, and 47.985 µg (Figure 3). And, 72 h after envenomation, only the guinea pig challenged with 17.795 µg of venom showed an increase in serum CK levels.

Of the animals that showed an increase in CK levels, only 2 also showed an increase in the cardiac fraction CK-MB, in relation to the reference interval: those inoculated with 76.775 µg after 24 h and 17.795 µg after 72 h.

The correlation analysis between the dose of *L. intermedia* venom applied and the serum level of CK and CK-MB (Table 2) did not reveal a statistically significant relationship between them 24 and 72 h after poisoning ($P > 0.05$) indicating no relationship between the variables during this period. Although the analysis indicated a perfect positive correlation between venom dose and serum CK levels after 72

h (R = 1.000), the result was not statistically significant (*P* = 0.714), suggesting that the observed association may have occurred by chance. The correlation between venom dose and serum CK-MB levels after 24 h was weak and non-significant (R = 0.200; *P* = 0.613), showing no consistent association between the variables during this period. After 72 h, there was also no significant correlation between the venom dose and serum CK-MB levels after 72 h (R = -0.029; *P* = 1.000), indicating no association between the variables during this period.

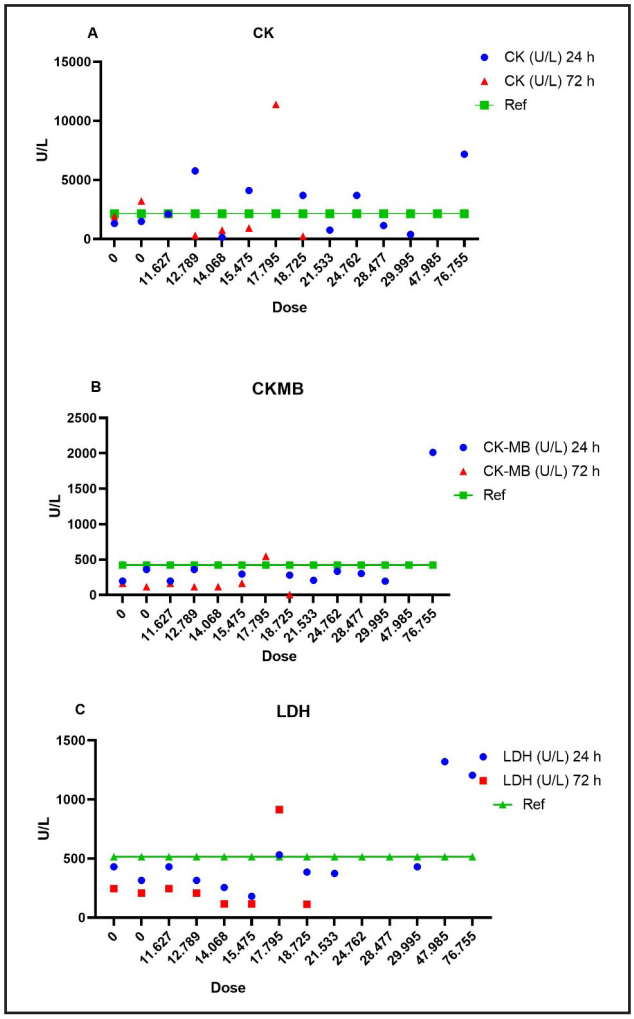

Figure 3. Serum levels of CK (U/L), CK-MB and LDH of *Cavia porcellus* submitted to different doses of *Loxosceles intermedia* venom, which showed a significant increase in relation to the reference value (red dotted line) after 24 and 72 h.

Two animals showed an increase in Troponin I in relation to the reference values stipulated by Life Diagnostics® in 2022 (0.2 - 12.5 ng/mL), both after the application of the venom: the animals submitted to the medium dose of 76.775 µg after 24 h and the low dose of 18.725 µg after 72 h. In addition, the animals inoculated with 18.725 µg, 24.762 µg, 28.477 µg and 76.775 µg showed higher levels of Troponin I and total CK, suggesting the occurrence of a possible cardiotoxic effect. A moderate but non-significant significant positive correlation was observed between the venom dose and serum Troponin I levels at 24 h (*P* = 0.700; *P* = 0.233), suggesting possible initial cardiac damage proportional to the dose.

Changes in serum LDH levels were observed in the animals inoculated with low and medium doses (17.795 µg, 47.985 µg and 76.755 µg), 24 h after the experimental envenomation and after 72 h in the animal inoculated with the low dose of 17.795 µg. A moderate positive correlation was observed between the dose and LDH levels at 24 h (R = 0.564), but without statistical significance (*P* = 0.096). After 72 h, the correlation was negative and non-significant (R = -0.371; *P* = 0.497), indicating no reliable association in this period. The correlation analysis between the dose of *L. intermedia* venom applied and the serum LDH level did not reveal a statistically significant relationship between them 24 and 72 h after envenomation (*P* > 0.05).

Regarding NT-proBNP, all the values found were below the test's lower limit of quantification (< 500 pmol/L). Likewise, the serum D-dimer concentration values found in the guinea pigs in this experiment were below the minimum quantification limit (<0.1 µg/mL) at the times evaluated.

Anatomical and histopathological changes

The animal challenged with 24.762 µg of *L. intermedia* venom showed a macroscopic increase in the volume of the heart, which had a thickened surface with a gelatinous and shiny appearance, suggestive of generalized edema and congestion. Microscopically, it was possible to observe marked diffuse cardiac congestion. In the animal challenged with 210 µg, the organ was macroscopically congested, increased in volume and dark red in color. Microscopically, moderate extensive focal hemorrhage was identified in the left atrium. The other guinea pigs challenged with *L. intermedia* venom showed no alterations under light microscopy (Figure 4).

Ultrastructural microscopy

The microstructural evaluation of the cardiac skeletal fibers of guinea pigs challenged with 12.789 µg showed the presence of multiple autophagic vacuoles interspersed with myofibrils in the sarcolemma and mitochondria with dilated and paraconcentric mitochondrial crests (Figure 5).

Table 2. Results of the correlation analysis between the dose of *L. intermedia* venom applied and the serum level of CK, CK-MB, TnI and LDH 24 h and 72 h after envenomation.

Correlation	N	R	*P*-value
Dose vs. CK (U/L) 24 h	12	0.006	1.000
Dose vs. CK (U/L) 72 h	8	-0.200	0.714
Dose vs. CK- MB (U/L) 24 h	11	0.2	0.613
Dose vs. CK -MB (U/L) 72 h	8	-0.029	1.000
Dose vs. Troponin I 24 h	7	0.7	0.233
Dose vs. Troponin I 72 h	5	-0.5	1
Dose vs. LDH (U/L) 24 h	12	0.4028	0.194
Dose vs. LDH (U/L) 72 h	8	-0.3713	0.3627

CK: creatine phosphokinase; CK-MB: creatine phosphokinase MB; TnI: Troponin I; LDH: lactate dehydrogenase.

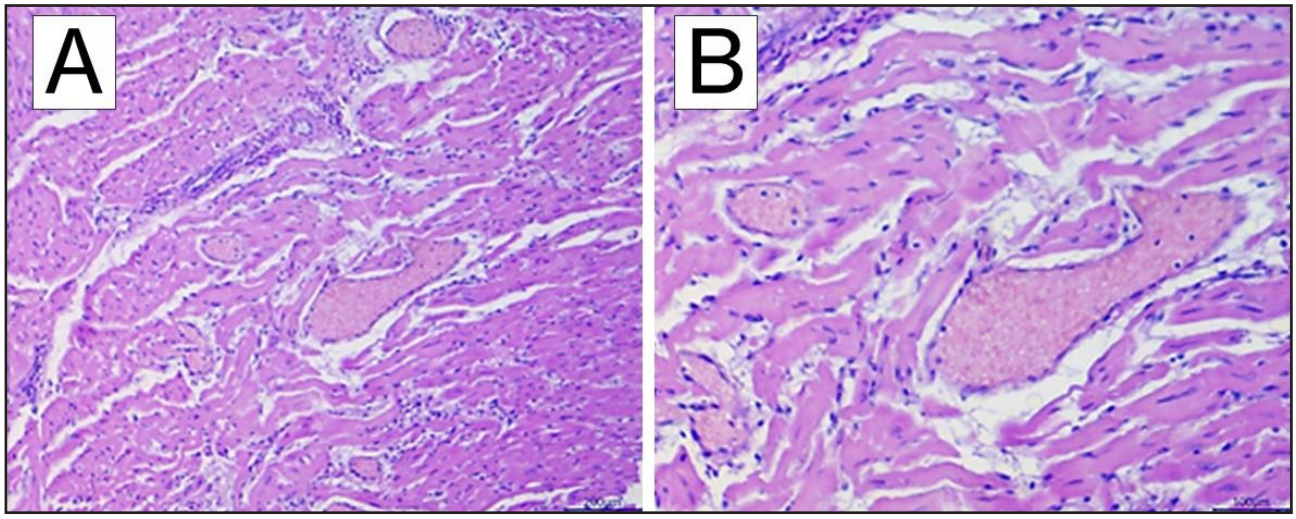

Figure 4. Cardiac photomicroscopies of *Cavia porcellus* inoculated with 24.762 µg of *Loxosceles intermedia* venom (animal 8), after HE staining. Note the marked diffuse congestion. [A: 200x / B: 400x].

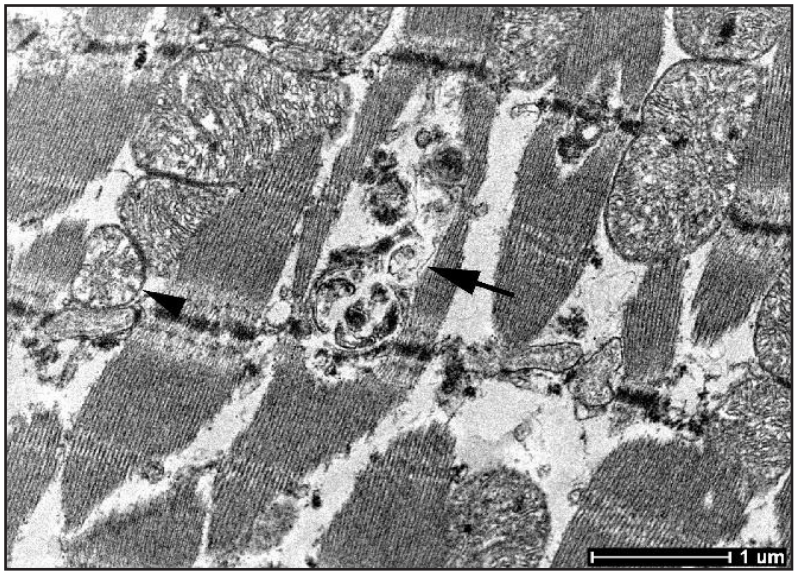

Figure 5. Ultramicrograph of myocardium. Multiple autophagic vacuoles interspersed with myofibrils in the sarcolemma (arrow) and mitochondria with dilated and paraconcentric mitochondrial crests (arrowhead). [Bar = 1 µm].

DISCUSSION

This study provides evidence of the cardiotoxic potential of *L. intermedia* venom in guinea pigs. The electrocardiogram is an essential diagnostic test in the assessment of cardiovascular disorders, especially when associated with other clinical and laboratory findings, and is widely used to identify heart rates, rhythm abnormalities, intraventricular conduction changes, and signs of myocardial ischemia [10]. Significant changes in electrocardiographic tracings were observed, corroborating the presence of cardiac dysfunctions associated with *L. intermedia* envenomation. Thus, the combination of electrocardiographic data with biochemical biomarkers strengthens the evidence of the cardiotoxic effect of *L. intermedia* venom and proves the development of cardiac dysfunction after envenomation.

The increase in serum levels of CK and CK-MB in guinea pigs challenged with low and medium doses of *L. intermedia* venom signals the occurrence of skeletal muscle and cardiac lesions after envenomation. These enzymes are widely used to diagnose and monitor cardiac disorders and are mainly associated with myocardial lesions [2]. This observed increase in CK levels may be related to the myotoxic action of the venom, which directly affects muscle fibers [7]. However, given that CK is a non-specific marker of muscle damage, these increases can be attributed to other factors. As for CK-MB, despite being more associated with the myocardium, it still has limitations in terms of its cardiac specificity, especially in animal models [2]. In addition, Spearman's correlation tests did not show statistically significant associations between the dose of venom and the serum levels of these markers in any of the periods analyzed, which limits the possibility of establishing a direct dose-response relationship through this test.

However, the significant increases observed in serum CK and CK-MB levels indicate that *L. intermedia* venom can cause generalized muscle damage, including possible damage to the heart muscle, although these markers are limited in their specificity. Corroborating these results, the increase in Troponin I, a specific "gold standard" biomarker for myocardial damage, especially up to 24 h after inoculation, when it reaches its maximum concentration in the blood, reinforces the suspicion that the venom exerts a deleterious cardiac effect, since during this period it was possible to observe a moderate positive correlation between the dose of venom inoculated and Troponin I levels, even though the association was not statistically significant.

In all the analysis carried out, the same pattern observed in the Troponin I dosage was identified in the LDH assessment. After 24 h of envenomation, a moderate positive correlation was also identified between the dose of *L. intermedia* venom and serum LDH levels after 24 h, and after 72 h, this same correlation became weak and, which may be related to the partial resolution of the lesions or the dynamics of enzyme degradation over time. LDH is a highly nonspecific enzyme and is considered a systemic biomarker of the organism as it is widely distributed in various cell types. Despite being a non-specific marker, its dosage contributes in a complementary way to the interpretation of the biochemical profile in contexts of tissue injury [23].

Although the dosage of NT-proBNP, an important marker of heart failure and ventricular wall stress, was below the limit of quantification, suggesting no ventricular overload [11,17], these isolated findings do not rule out the possibility of heart damage, especially considering the alterations detected on the ECG. Furthermore, it is important to note that the specificity and validation of the test for guinea pigs, as well as the short half-life of NT-proBNP, may limit its sensitivity for detecting damage at different times after envenomation.

It is important to note that animals that received high doses died quickly, probably due to the development of disseminated intravascular coagulopathy and shock a few hours after venom inoculation, causing occlusion of dermal venules and arterioles, as observed in humans and rabbits, aggravating tissue hypoxia [16,18,24]. Such an early and rapid evolution of this envenomation is unusual, according to the literature review. However, it is known that the mechanism of action of the venom is multifactorial and still not fully understood.

In addition, D-dimer, a specific product of the degradation of fibrin clots, was measured, since the occurrence of coagulopathies is a rare complication, although it has been described in systemic loxoscelism [9,20]. However, the levels of D-dimer

in the guinea pigs did not show significant elevations, indicating the absence of serious coagulation disorders associated with *L. intermedia* envenomation at the dosages tested in this experiment. In a retrospective study conducted at the Vital Brazil Hospital with human patients treated between 2004 and 2006, 53.5% showed elevated D-dimer levels 1 week after the accident, and 25% 2 weeks after, although none were diagnosed with disseminated intravascular coagulation (DIC) [13]. Considering these data and the fact that, in the present study, the animals were euthanized only 3 days after the venom inoculation, it is possible that the observation time was insufficient for changes in D-dimer levels to become detectable. Therefore, the possibility of late development of hypercoagulability alterations cannot be completely ruled out.

At the microscopic level, it was possible to observe marked diffuse cardiac congestion. In the animal challenged with a high dose of 210 μg, the organ was congested, with increased volume and a dark red color. Microscopically, moderate extensive focal hemorrhages were identified in the left atrium. The other guinea pigs challenged with *L. intermedia* venom showed no alterations under light microscopy. After microstructural evaluation, multiple autophagic vacuoles were observed interspersed with the myofibrils in the sarcolemma, indicating the occurrence of an active autophagic process, which can lead to the development of tissue necrosis. In addition, the mitochondria had dilated mitochondrial crests and were organized in a paraconcentric manner, suggesting cellular functional impairment [16]. These findings corroborate the hypothesis that the venom has a direct effect on cardiac tissue, promoting structural and metabolic dysfunction at a cellular level, in agreement with the results of the cardiac biochemical profile, which showed an increase in CK, CK-MB and troponin I, in addition to the electrocardiographic alterations found. This clearly shows the potential cardiotoxic effect of *L. intermedia* venom, which, even at different doses, has been shown to cause damage to cardiac fibers, reinforcing the seriousness of the cardiac damage caused by this venom.

CONCLUSIONS

This study provides robust evidence of the cardiotoxic potential of *L. intermedia* venom, confirming that its toxins can cause significant damage to the cardiovascular system. The joint analysis of biochemical, electrocardiographic, and histopathological results demonstrated that the venom directly affects cardiac function, causing everything from arrhythmias to lesions in cardiac muscle fibers, as evidenced by electron microscopy. The increase in injury biomarkers such as CK, CK-MB and troponin I, associated with the changes observed in the ECG and microstructural analyses, provides a clearer understanding of the cardiac impact caused by the venom. In addition, the results demonstrate that *L. intermedia* venom can cause cardiac lesions at different doses, with important implications for understanding systemic loxoscelism and its effects on the cardiovascular system.

MANUFACTURERS

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***Ethical approval*.** This study was conducted in accordance with ethical principles, respecting animal welfare, and approved by the Ethics Committee for the Use of Animals (CEUA) of the Federal University of Minas Gerais, CEUA protocol No 131/2020.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1 **Appel M.H., da Silveira R.B., Chaim O.M., Paludo K.S., Silva D.T., Chaves D.M., da Silva P.H., Mangili O.C., Senff-Ribeiro A., Gremski W., Nader H.B. & Veiga S.S. 2008.** Identification, cloning and functional characterization of a novel dermonecrotic toxin (phospholipase D) from brown spider (*Loxosceles intermedia*) venom. *Biochimica et Biophysica Acta*. 1780: 167-178. DOI: 10.1016/j.bbagen.2007.11.007.

2 **Bachmaier K., Mair J., Offner F., Pummerer C. & Neu N. 1995.** Serum cardiac troponin T and creatine kinase–MB elevations in murine autoimmune myocarditis. *Circulation*. 92: 1927-1932. DOI: 10.1161/01.cir.92.7.1927.

3 **Binford G.J. & Wells M.A. 2003.** The phylogenetic distribution of sphingomyelinase D activity in venoms of Haplogyne spiders. *Comparative Biochemistry and Physiology Part* B. 135: 25-33. DOI: 10.1016/s1096-4959(03)00045-9.

4 **Botelho A.F., Oliveira M.S.D., Soto-Blanco B. & Melo M.M. 2016.** Computerized electrocardiography in healthy conscious guinea pigs (*Cavia porcellus*). *Pesquisa Veterinária Brasileira*. 36: 1203-12083.

5 **Chávez-Olortegui C., Zanetti V.C., Ferreira A.P., Minozzo J.C., Mangili O.C. & Gubert I.C. 1998.** ELISA for the detection of venom antigens in experimental and clinical envenoming by *Loxosceles intermedia* spiders. *Toxicon*. 36: 563-569. DOI: 10.1016/s0041-0101(97)00159-1.

6 **De Chiara M., Scatteia A., Ferrante G. & Dellegrottaglie S. 2024.** Uncommon cardiac complications following *Loxosceles rufescens* spider bite: a case report with diagnostic insights. *Journal of Cardiovascular Magnetic Resonance*. 26: 450-453.

7 **Dias-Lopes C., Felicori L., Guimarães G., Gomes E.R., Roman-Campos D., Duarte H., Damasceno D., Martins M., Kalapothakis E., Almeida A.P., Granier C., Cruz J.S., Guatimosim S. & Chávez-Olórtegui C. 2010.** Cardiotoxic effects of *Loxosceles intermedia* spider venom and the recombinant venom toxin rLiD1. *Toxicon*. 56: 1426-1435. DOI: 10.1016/j.toxicon.2010.08.008.

8 **Gremski L.H., da Justa H.C., Polli N.L.C., Schluga P.H.C., Theodoro J.L., Wille A.C.M., Senff-Ribeiro A. & Veiga S.S. 2022.** Systemic loxoscelism, less frequent but more deadly: The involvement of phospholipases d in the pathophysiology of envenomation. *Toxins*. 15: 17. DOI: 10.3390/toxins15010017.

9 **Gremski L.H., Trevisan-Silva D., Ferrer V.P., Matsubara F.H., Meissner G.O., Wille A.C., Vuitika L., Dias-Lopes C., Ullah A., de Moraes F.R., Chávez-Olórtegui C., Barbaro K.C., Murakami M.T., Arni R.K., Senff-Ribeiro A., Chaim O.M. & Veiga S.S. 2014.** Recent advances in the understanding of brown spider venoms: From the biology of spiders to the molecular mechanisms of toxins. *Toxicon*. 83: 91-120. DOI: 10.1016/j.toxicon.2014.02.023.

10 **Islam M.S, Kalmady S.V., Hindle A., Sandhu R., Sun W., Sepehrvand N., Greiner R. & Kaul P. 2024.** Diagnostic and prognostic electrocardiogram-based models for rapid clinical applications. *The Canadian Journal of Cardiology*. 40: 1788-1803. DOI: 10.1016/j.cjca.2024.07.003.

11 **Kaczyñska A., Pelsers M.M., Bochowicz A., Kostrubiec M., Glatz J.F. & Pruszczyk P. 2006.** Plasma heart-type fatty acid binding protein is superior to troponin and myoglobin for rapid risk stratification in acute pulmonary embolism. *Clinica Chimica Acta*. 371: 117-123. DOI: 10.1016/j.cca.2006.02.032.

12 **Langner T.R., Ganatra H.A., Schwerdtfager J., Stoecker W. & Thornton S. 2021.** Viscerocutaneous loxoscelism manifesting with myocarditis: a case report. *The American Journal of Case Reports*. 22: e932378. DOI: 10.12659/AJCR.932378.

13 **Malaque C.M, Santoro M.L., Cardoso J.L., Conde M.R., Novaes C.T., Risk J.Y., França F.O., de Medeiros C.R. & Fan HW. 2011.** Clinical picture and laboratorial evaluation in human loxoscelism. *Toxicon*. 58: 664-671. DOI: 10.1016/j.toxicon.2011.09.011.

14 **Manning P.J., Wagner J.E. & Harkness J.E. 1994.** Biology and diseases of guinea pigs. In: *Laboratory Animal Medicine*. 2nd edn. Orlando: Academic Press, pp.149-177.

15 **Navarro-Martinez D.A., Berarducci J., Armenta-Moreno J.I., Armendariz-Ferrari J.C., Garcia-Cardenas M., Fernandez-Badillo V. & Espinola-Zavaleta N. 2022.** Kounis Syndrome: Acute coronary syndrome induced by a *Loxosceles laeta* bite in a 9-year-old boy. *CASE*. 6: 450-453. DOI: 10.1016/j.case.2022.09.001.

16 **Ospedal K.Z., Appel M.H., Fillus Neto J., Mangili O.C., Sanches Veiga S. & Gremski W. 2002.** Histopathological findings in rabbits after experimental acute exposure to the *Loxosceles intermedia* (brown spider) venom. *International Journal of Experimental Pathology*. 83: 287-294. DOI: 10.1046/j.1365-2613.2002.00241.x.

17 **Pruszczyk P. 2005.** N-terminal pro-brain natriuretic peptide as an indicator of right ventricular dysfunction. *Journal of Cardiac Failure*. 11: S65-S69. DOI: 10.1016/j.cardfail.2005.04.016.

18 **Rosen J.L, Dumitru J.K., Langley E.W. & Meade Olivier C.A. 2012.** Emergency department death from systemic loxoscelism. *Annals of Emergency Medicine*. 60: 439-441. DOI: 10.1016/j.annemergmed.2011.12.011.

19 **Senff-Ribeiro A., Silva P.H., Chaim O.M., Gremski L.H., Paludo K.S, Bertoni da Silveira R., Gremski W., Mangili O.C. & Veiga S.S. 2008.** Biotechnological applications of brown spider (*Loxosceles genus*) venom toxins. *Biotechnology Advances*. 26: 210-218. DOI: 10.1016/j.biotechadv.2007.12.003.

20 **Silva P.H., Silveira R.B., Appel M.H., Mangili O.C., Gremski W. & Veiga SS. 2004.** Brown spiders and loxoscelism. *Toxicon*. 44: 693-709. DOI: 10.1016/j.toxicon.2004.07.012.

21 **Silveira R.B., Santos Filho J.F., Mangili O.C., Veiga S.S., Gremski W., Nader H.B. & von Dietrich C.P. 2002.** Identification of proteases in the extract of venom glands from brown spiders. *Toxicon*. 40: 815-822. DOI: 10.1016/s0041-0101(02)00078-8.

22 **Theodoro J.L., da Justa H.C., de Caires Schluga P.H., Fischer M.L., Minozzo J.C., Gremski L.H. & Veiga S.S. 2024.** Subtranscriptome analysis of phospholipases D in *Loxosceles* venom glands: Confirmation of predominance, intra-species diversity, and description of novel isoforms. *International Journal of Biological Macromolecules*. 280: 136108. DOI: 10.1016/j.ijbiomac.2024.136108.

23 **Thrall M.A., Baker D.C., Campbell T.W., DeNicola D., Fettman M.J., Lassen E.D., Rebar A. & Weiser G. 2004.** *Veterinary Hematology and Clinical Chemistry*. Philadelphia: Lippincott Williams & Wilkins, 518p.

24 **Zanetti V.C., Silveira R.B., Dreyfuss J.L., Haoach J., Mangili O.C., Veiga S.S. & Gremski W. 2002.** Morphological and biochemical evidence of blood vessel damage and fibrinogenolysis triggered by brown spider venom. *Blood Coagulation & Fibrinolysis*. 13: 135-148. DOI: 10.1097/00001721-200203000-00009.