The content of inflammatory, antiphlogistic and stress biomarkers in heart after *Leiurus macroctenus* envenomation

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Ethics approval: all experiments on animals were performed in the compliance with international principles of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986). The study was approved by the Ethical Committee of Taras Shevchenko National University of Kyiv (protocol № 2 approved 19.08.2021).

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Abstract

Scorpions belonging to the species *Leiurus macroctenus* are known to be very dangerous, but the effect of their venom on cytokine and stress biomarker levels in the heart is still poorly understood. The aim of this study was to analyse the pro-inflammatory, anti-inflammatory and stress biomarkers content in rat heart after *L. macroctenus* envenomation. A significant increase in the content of both pro-inflammatory cytokines: tumour necrosis factor-α (TNF-α), interleukins-1β, -8, -6 (IL-1β, IL-8, IL-6), and anti-inflammatory cytokines: interferon-γ (IFN-γ), interleukins-4, -10 (IL-4, IL-10), as well as an increase in transcription factor Nuclear Factor-kappa B (NF-κB), Hypoxia-Inducible Factor-1 alpha (HIF-1α), and the heat shock proteins (HSP60 and HSP70) content were found in heart tissue. All this may indicate that *L. macroctenus* envenomation may cause significant destruction of the cellular microenvironment in the heart with certain changes in the innate immune response, leading to systemic poisoning. Moreover, a decrease in the level of all analysed indicators was detected 72 hours after the introduction of the venom, which may be a sign of the beginning of reparative processes in the organ.

Introduction

Scorpions belonging to the genus *Leiurus* (also known as "deathstalker") are well known for their dangerous venom.[1] *Leiurus macroctenus* is a recently identified species that differs from other *Leiurus* species in morphological and morphometric parameters.[2]

The number of scorpion poisoning cases is increasing year by year. Every year, about 1 million cases of scorpion envenomation and almost 3,250 deaths from scorpion sting are recorded.[3] The toxic components of scorpion venom cause, in addition to local symptoms, the development of severe neurological disorders, damage to the cardiovascular system, etc. Under these conditions, the main causes of death are heart failure and pulmonary edema.[4,5]

It is known that the venoms of even closely related scorpion species can differ significantly in both their quantitative and sometimes qualitative composition.[6] Scorpion venoms are mostly composed of inorganic salts, amino acids, nucleotides, lipids, biogenic amines, serotonin, histamine enzymes, and peptides.[6,7] The enzymes present in the venom enhance the poisoning process and venom distribution by disrupting the extracellular matrix.[7,8] At the same time, metallo- and serine proteases activate latent forms of toxins and endogenous signalling molecules, and also modulate the production of cytokines.[9] Enhanced enzymatic activity during envenomation leads to increased tissue permeability and provides a

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systemic inflammatory response, as venom toxins can easily spread throughout all organs and tissues.[9]

In addition, neurotoxins from scorpion venom can affect the secretion of neurotransmitters, hormones, and cytokines, water-salt balance, and blood pressure, leading to cardiovascular, pulmonary, and gastrointestinal complications.[10,11] At the same time, nondisulfide-bridged peptides from venom are small amphipathic peptides that interact nonspecifically with membranes, exhibiting antibacterial, antiviral and antifungal activity. Some of them exhibit immunomodulatory and bradykinin-potentiating activities. This suggests that certain components of scorpion venom have biomedical properties, and this is also attracting the attention of scientists.[8,12-14]

To date, little is known about the role of inflammation in the pathogenesis of *L. macroctenus* envenomation. There are also insufficient data on the levels of pro-inflammatory, such as (tumour necrosis factor-α (TNF-α), interleukins-1β, -8, -6 (IL-1β, IL-8, IL-6) and anti-inflammatory cytokines, such as (interferon-γ (IFN-γ), interleukins-4, -10 (IL-4, IL-10) and their regulators – transcription factors and growth factors, such as (transcription factor Nuclear Factor-kappa B (NF-κB), Hypoxia-Inducible Factor-1 alpha (HIF-1α), and the heat shock proteins (HSP60 and HSP70) in damaged organs in this condition. Thus, the aim of this study was to analyse the pro-inflammatory, anti-inflammatory and stress biomarkers content in heart after *L. macroctenus* envenomation.

Materials and Methods

Ethics

The case-control study with animals followed the international recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and was confirmed by the Bioethical Commission of the University of Kyiv (protocol № 2 approved 19.08.2021).

Scorpion collection and maintenance

Scorpions were maintained separately in transparent plastic boxes (10 × 5 × 5 cm) layered with sand («Desert Sand», Exo Terra, HAGEN Deutschland GmbH & Co, Hagen, Germany) by 1 cm. Water bowls with drinking water, which were refilled every week, were placed in the centre of each box. All animal containers were kept in constant conditions (25-35°C, 50-60% humidity, natural lighting conditions). Proper aeration was reached by numerous holes in the container. Specimens were fed upon one *Shelfordella lateralis* cockroach once per week, in case of refusal of food, cockroach was taken away in 2 days after feeding. Once per month, containers were cleaned of cockroach remnants.

Venom milking

The procedure of venom collection was performed according to Ozkan and Filazi's method.[15] The method for determination of acute lethal dose-50 (LD50) levels of venom in rats, was reported for *Androctonus crassicauda* species, and modified by Yaqoob.[16] Scorpion fixation was followed by electrode pointing to cephalotorax and telson. Electric current with intensity of 24 V was applied for 5 s to the base of telson, while opposite edge of telson was pointed to the sterile phial. Depending on the amount of collected venom, the number of electrode-scorpion contacts varied, up to 10. The interval between milking acts was ranged at 2 weeks. The collected venom samples were stored at -20°C.

Study design

Albino male rats (n=70 total; age 2 months; weight 180 g±3 g) participated in the study. Performing the experiment, rats were randomly divided into 2 groups: a group of 60 albino male rats was injected intramuscularly with 0.5 mL venom solution, dissolved in saline solution (0.9%). Despite the LD50 value, only 20 out of 60 animals died, so the total number of rats included in the study was 40: exactly 10 animals were taken for each time point (1 h, 3 h, 24 h and 72 h). Control group (10 rats) was injected with 0.5 mL saline solution (0.9%) alone. To estimate dynamic changes of analysed parameters, rats were sacrificed by cervical dislocation after 1 h, 3 h, 24 h, and 72 h following the venom injection. The hearts were immediately collected.

Preparation of homogenates of hearts

Rats hearts were isolated and homogenized at 4°C. Homogenization was conducted using 50 mM Trishydroxymethyl aminomethan-HCl (Tris-HCl) (pH 7.4) buffer solution with 140 mM NaCl and 1 mM ethylenediaminetetraacetic acid (EDTA) addition. The volume of used buffer was five times higher (in grams) than isolated organs' mass. Crude homogenate was centrifuged at 600 g for 15 min with further supernatant collection and centrifugation at 15,000 g for 15 min. Obtained homogenate aliquots were frozen in liquid nitrogen.

Protein content quantification

Protein concentrations were measured by the Bradford assay.[17]

Enzyme-linked immunosorbent assay

The determination of the content of parameters in tissue homogenates was carried out using the method of immune-enzymatic analysis in 96-well microplates with sorption capacity according to the standard method for soluble proteins.[18] The antigen was diluted with 0.05 M Tris-HCl buffer (pH 7.4) to a concentration of one μg/mL and incubated in the wells of the plates at 4ºC overnight. After incubation, to remove unbound antigen, the wells were washed with immobilization buffer. Blocking of nonspecific binding sites was carried out by incubation with 5% skim milk solution for 1 hour at 37ºC. After incubation, the wells were washed with working buffer containing 0.1% Tween-20 and incubated with a solution of corresponding primary antibodies (mouse monoclonal) against NF-κB, HIF-1α, HSP60, HSP70, IFN-γ, TNF-α, and IL-1β, -4, -6, -8, -10 (Santa Cruz Biotechnology, Inc., Dallas, Texas, USA), the dilution of which was 1:3000 for one hour at 37ºC. Further washing was performed with working buffer containing 0.1% Tween-20 and incubated with secondary antibodies (anti-mouse) conjugated to horseradish peroxidase (Sigma-Aldrich, St. Louis, MO, USA) (dilution 1:3000) for one hour at 37ºC. After the incubation, the wells were washed again with buffer containing 0.1% Tween-20 and incubated with the substrate o-phenylenediamine (OPD), at a concentration of 0.4 mg/mL diluted in 0.05 M phosphate-citrate buffer with the addition of 30% H_2O_2 to visualize the binding of secondary antibodies. The peroxidase reaction was stopped by adding 100 μL of 1 M H_2SO_4 after 10 min. The optical absorbance was measured at a wavelength of 492 nm on a microplate spectrophotometer (μQuantTM, BioTek Instruments, Inc, USA).

Statistics

The results were tested for normal distribution using the Shapiro-Wilk test. Homogeneity of variance was assessed using Bartlett's test for equality of variances. Thereafter, the significance of differences between the means of experimental groups was determined by one-way analysis of variances (ANOVA) with Turkey's multiple comparisons test,[19] performed in GraphPad Prism 9 (GraphPad Software Inc., Boston, USA). Values, present in tables and figures are expressed as mean±Standard Error of Mean (SEM). When $p<0.05$, the differences between groups were considered statistically significant.

Results

At the first stage of our molecular-biological research, the results showed a significant elevation in pro-inflammatory cytokines content in the hearts of rats after *L. macroctenus* envenomation compared to the control group: TNF-α (1.1-, 1.2-, 1.3- and 1.1-fold increment ($p\leq0.001$) in 1-72 h of envenomation, respectively), IL-1β (1.2-, 1.4-, 1.6- and 1.3-fold increase ($p\leq0.001$) in 1-72 h after injection, respectively), IL-8 (1.1-, 1.1-, 1.2- and 1.1-fold increment, ($p\leq0.001$), in 1-72 h of envenomation, respectively) and IL-6 (1.1-, 1.3-, 1.4- and 1.1-fold increase, ($p\leq0.001$), in 1-72 h after injection, respectively) (Figure 1). Moreover, peak levels were observed in the 24 hours after venom injection, with further decrement of TNF-α, IL-1β, IL-8 and IL-6 content (Figure 1).

The results of the further experimental studies have shown that envenomation had impact on anti-inflammatory cytokines content in the hearts of rats compared to the control group: IFN-γ content was higher by 1.1, 1.3, 1.5 and 1.1 times ($p\leq0.001$) in 1-72 h after injection, respectively; IL-4 content increased by 1.1, 1.2, 1.2 and 1.1 times ($p\leq0.001$) in 1-72 h of envenomation, respectively; and at the same time, IL-10 content was higher by 1.1, 1.1, 1.2 and 1.1 times ($p\leq0.001$) in 1-72 h of after venom injection, respectively (Figure 2). Also, we demonstrated that generally, peak levels were observed in the 24 hours after venom injection, with further decrease of IFN-γ, IL-4 and IL-10 content (Figure 2).

We also found elevated content of transcription factor NF-κB (1.1-, 1.2-, 1.3- and 1.1-fold increment, ($p\leq0.001$), in 1-72 h of envenomation, respectively), hypoxic factor HIF-1α (1.1-, 1.1-, 1.2- and 1.1-fold increase, ($p\leq0.001$), in 1-72 h after injection, respectively) and the heat shock proteins HSP60 and HSP70 (1.1-, 1.2-, 1.1- and 1.1-fold increment, ($p\leq0.001$, $p\leq0.01$), and 1.1-, 1.1-, 1.2- and 1.1-fold increase, ($p\leq0.001$), in 1-72 h after injection, respectively) (Table 1). For these indicators, the highest levels were observed three hours (for HSP60) and 24 hours (for NF-κB, HIF-1α and HSP70) after venom injection, with a subsequent decrease in the content of NF-κB, HIF-1α, HSP60 and HSP70 (Table 1).

Discussion

The toxic effect of *L. macroctenus* venom on the heart has been demonstrated in previous study.[5] It was found out a significant increase in the width of cardiomyocytes and the area of haemorrhage zones in the myocardium after venom injection.[5] Thus, cardiomyocytes showed signs of pathological changes already in the first hour after the venom exposure, while the width of cardiomyocytes was already significantly greater by the third hour of envenomation.[5]

Our results showed a significant elevation in pro-inflammatory

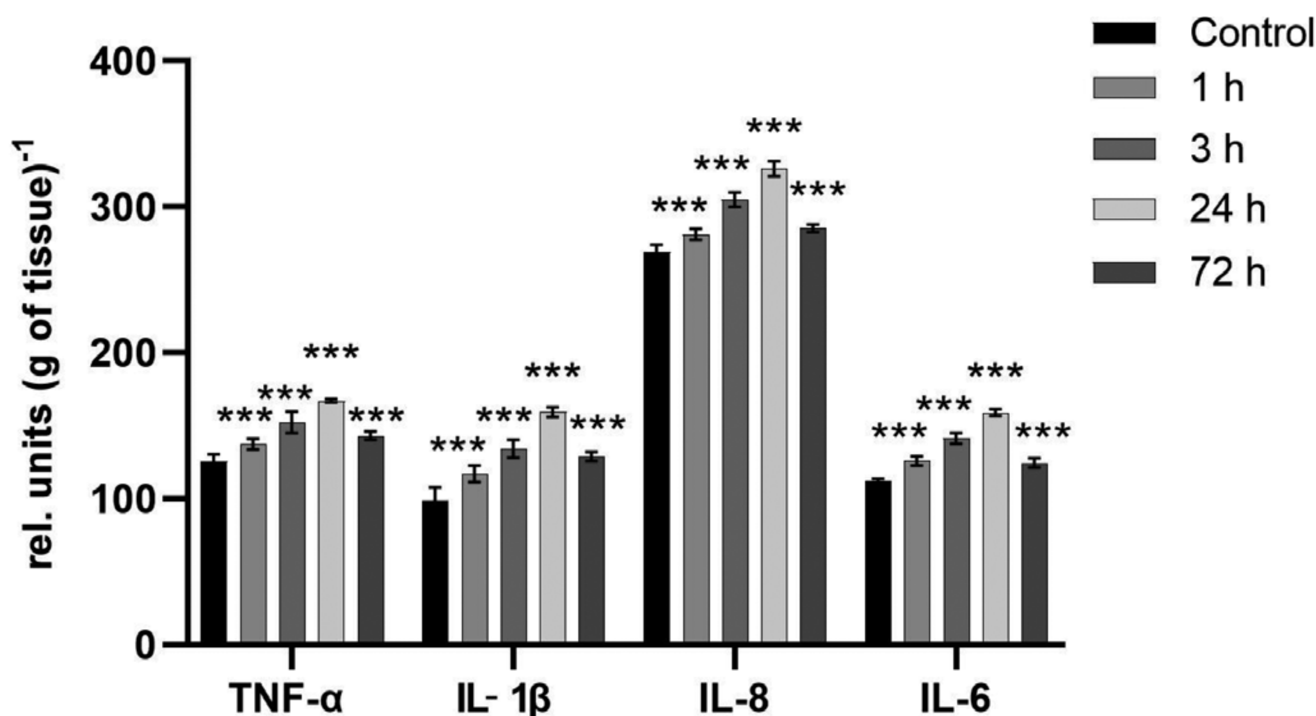

Figure 1. Pro-inflammatory cytokines tumour necrosis factor-α (TNF-α), and interleukins-1β, -8, -6 (IL-1β, IL-8, IL-6) content in the hearts of rats after *Leiurus macroctenus* envenomation. Results are presented as mean±Standard Error of Mean (SEM) (n=10). ***$p\leq0.001$ *vs.* control.

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cytokines content in the hearts of rats after *L. macroctenus* envenomation compared to the control group (Figure 1). Cytokines produced by immune cells regulate the immune response, causing tissue damage, and mediating complications of the inflammatory process.[9,11] Pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α are responsible for protection against exogenous pathogens, but their harmful effects have been shown when overproduced.[20] A number of researches have reported high levels of pro-inflammatory cytokines (IL-1α, IL-1β, TNF-α) in the serum of both humans bitten by scorpions and experimental animals envenomed by various species of scorpions.[11,20,21] In studies of the effects of venom on skeletal muscle, it was shown that injury increased the expression levels of TNF-α and IL-1β, which further activated the synthesis of IL-6 and IL-8 by the vascular endothelium. These cytokines induced the migration of neutrophils to the injury site during the first few hours of the acute inflammatory response, with a peak between 4 and 6 hours. In turn, neutrophils provided the production of pro-inflammatory cytokines, reactive oxygen species and proteinases, thereby locally damaging the tissues after venom administration.[22] In addition, neutrophils secrete chemoattractants that direct blood monocytes to the site of inflammation, causing macrophage infiltration, which begins 24-48 hours after envenomation. Overexpression of pro-inflammatory cytokines, including IFN-γ, induces the synthesis of pro-inflammatory M1 macrophages.[22]

Peak levels of M1 macrophage infiltration have been shown to occur 1-3 days after injury. At the same time, macrophage-mediated clearance of apoptotic neutrophils induces secretion of pleiotropic cytokines and IL-10, leading to the formation of anti-inflammatory M2 macrophages. So, after reaching the maximum concentration of M1 macrophages, the pro-inflammatory microenvironment can transform into an anti-inflammatory one, where there is a higher concentration of M2 macrophages producing IL-4 and -10, and, accordingly, a different expression pattern of these cytokines.[23,24] Thus, the results of our further experimental studies showed that envenomation led to an increase in anti-inflammatory cytokines content in the hearts of rats compared to the control group (Figure 2).

Studies analysing the effects of scorpion venom on myocardial cells have identified three main factors of cardiac dysfunction: adrenergic myocarditis, toxic myocarditis, and myocardial ischemia. Such acute effects developed within the first hour after envenoma-

Table 1. Transcription factor Nuclear Factor-kappa B (NF-κB), Hypoxia-Inducible Factor-1 alpha (HIF-1α), and heat shock proteins HSP60 and HSP70 content in the hearts of rats after *Leiurus macroctenus* envenomation.

Relative units·(g of tissue)$^{-1}$	Control	One hour	Three hours	24 hours	72 hours
NF-κB	153.29±1.64	167.33±4.85***	184.06±3.86***	198.74±2.85***	173.64±4.63***
HIF-1α	287.64±2.32	301.56±2.86***	317.38±2.96***	331.05±3.83***	302.53±4.95***
HSP60	176.39±3.29	189.42±1.37***	201.52±1.28***	192.64±4.97***	181.95±1.28**
HSP70	231.62±4.27	245.08±2.74***	258.08±3.04***	286.39±1.99***	253.65±0.96***

Results are presented as mean±Standard Error of Mean (SEM) (n=10). **$p<0.01$,***$p<0.001$ *vs.* control.

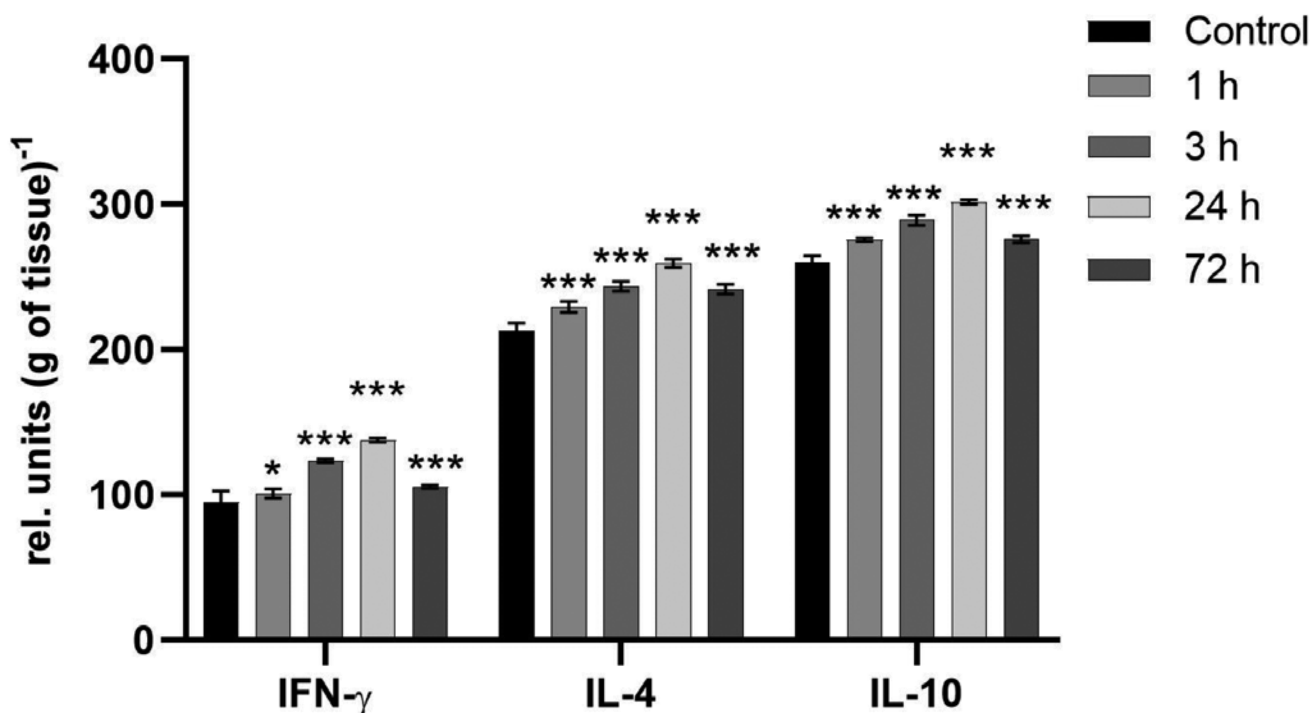

Figure 2. Anti-inflammatory cytokines interferon-γ (IFN-γ), and interleukins-4, -10 (IL-4, IL-10) content in the hearts of rats after *Leiurus macroctenus* envenomation. Results are presented as mean± Standard Error of Mean (SEM) (n=10). *$p<0.05$, ***$p<0.001$ *vs.* control.

tion.[5] After this, during cardiomyocytes regeneration, activation, proliferation and fusion of myogenic satellite cells occur with the formation of myotubes. On day 7, most of the damaged muscles are replaced by small regenerating fibers which acquire their normal diameter in a month. Cytokines, chemokines, and growth factors synthesized under inflammatory conditions are involved in the regulation of these processes.[23] In our work, an increase in the content of anti-inflammatory cytokines was found (with peak levels were observed in the 24 hours after venom injection) (Figure 2), which may indicate a change in the phenotype of the predominant macrophage population in cardiomyocytes, since changes in the cytokine expression pattern are an important indicator of this process.[25,26] We additionally analyzed the levels of transcription factors NF-κB and HIF-1α, as these proteins are important regulators of cytokine biosynthesis and may be useful for understanding the mechanisms of the changes we detected. We found a significant increase in their content after venom exposure (Table 1), the peak of which coincided in time with the maximum changes detected in cytokine levels (24 h).

NF-κB is one of the main transcription factors that is able to coordinate innate and adaptive immunity, the development of inflammatory reactions, as well as other processes, such as cell proliferation and differentiation, etc.[27] TNF-α, in addition to modulating the above mentioned molecular pathways, can, in turn, regulate the expression of NF-κB.[20,27] It is known that NF-κB also affects the classical signalling pathways of pro-inflammatory cytokines involved in the pathogenesis of the tissue response to *L. macroctenus* envenomation.[20]

Our observed increase in TNF-α and NF-κB levels in rat hearts after venom injection suggests a potential link between persistent inflammatory activity and immune response. It was also found that changes in NF-κB activity were directly associated with increased expression of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α.[20,28] In addition, certain cytokines, including TNF-α, can bind to the extracellular matrix, prolonging their biological activity in tissues and thereby inducing transcriptional changes involving the NF-κB signalling pathway.[29]

HIF-1α modulates the cellular response to hypoxia and may play a crucial role in the activation of immune cells during inflammation. It is involved in regulating the expression levels of many genes involved in extracellular matrix remodelling, vasodilation, and angiogenic pathways by modulating the transcription of a number of growth factors.[30] It is known that respiratory and cardiovascular complications after scorpion envenomation cause acute hypoxia and rapidly worsen respiratory status, which can lead to HIF-1 activation.[9,30] In addition, HIF-1 is able to regulate the immune response. Thus, under conditions of hypoxia and with the participation of HIF-1, the gene expression pattern of macrophages in hypoxic areas rapidly changes, activating the synthesis of numerous pro-inflammatory cytokines and chemokines.[30] So, the increased content of HIF-1α in rat heart homogenates may indicate that the immune system tried to adapt to envenomation by activating angiogenesis and corresponding tissue repair mechanisms. At the same time, a drop in the level of all studied indicators 72 h after injection may indicate the beginning of reparative processes in the organ.

In our work, the immune and cellular response to venom induced also the activation of HSPs: HSP60 and HSP70 (with the highest levels in 3 hours for HSP60, and 24 hours for HSP70 after venom injection) (Table 1). The main functions of HSP60 and HSP70 are chaperoning misfolded or unfolded polypeptides, protecting cells from the toxic effects of intracellular or extracellular stressors, and regulating immune and inflammatory responses through interaction with cytokines.[31] So, the observed increase in HSP60 and HSP70 content in the hearts of rats after *L. macroctenus* envenomation may indicate upregulation of the immune system in this pathological conditions. Thus, the increase in HSP60 and HSP70 in cardiac tissue after scorpion venom administration may indicate the activation of a cellular stress response. HSP60 and HSP70 function as «molecular chaperones,» protecting cells from the accumulation of denatured or misfolded proteins. Their expression increases significantly in response to hypoxia, toxic injury, cytokine stress, and calcium homeostasis disorders, factors that may be a direct consequence of the action of *L. macroctenus* venom components, which, in turn, may affect the ability to regulate cellular homeostasis properly.

Overall, our observed changes in the analysed parameters suggest that the immune state after venom administration is characterized by complex interactions in acute stress response pathways, where modulation of HSP levels reflects a shift from acute cellular stress to likely long-term immune adaptation with some reparative processes in the organ. [8,11,31-33]

In general, based on our studies, it can be concluded that the venom of *L. macroctenus* may potentially contain components with pro- and anti-inflammatory properties, and these components, in turn, are capable of causing a complex effect on cardiomyocytes.[5,8,33] Our results indicate the effect of venom on both the cytokine profile (pro-inflammatory cytokines: TNF-α, IL-1β, IL-8, IL-6, and anti-inflammatory cytokines IFN-γ, IL-4, IL-10) and the content of their regulators (transcription factor NF-κB, hypoxic factor HIF-1α, and the heat shock proteins HSP60 and HSP70) in rat heart homogenates, which confirms the appearance of corresponding changes in the innate immune response. Our findings may be useful for developing differentiated and more effective treatment strategies for the effects of these scorpion stings.[8,12-14,33]

Despite this, our data require additional studies due to following limitations of the study. Thus, assessment of serum cytokine levels at different time points after venom injection may help to identify the involvement of pro- and anti-inflammatory signalling pathways, as well as possible allergic reactions in the mechanisms of poisoning. Investigation of the effects of crude venom and its toxins on isolated immune cells may be useful to establish their potential influence on cytokine release by immune cells. Histological studies of heart tissue in 24 and 72 hours after the venom exposure may also complement the obtained molecular-biological data.

Conclusions

In this study the pro-inflammatory, anti-inflammatory and stress biomarkers content in heart after *L. macroctenus* envenomation were analysed. The following changes were found in the heart tissue: a significant increase in the content of both pro-inflammatory cytokines: TNF-α, IL-1β, IL-8, IL-6, and anti-inflammatory cytokines IFN-γ, IL-4, IL-10, as well as an increase in transcription factor NF-κB, hypoxic factor HIF-1α, and the heat shock proteins HSP60 and HSP70 content. All this may indicate that *L. macroctenus* envenomation cause significant destruction of the cellular microenvironment in the heart with certain changes in the innate immune response, leading to systemic poisoning. Whereas a tendency towards all indicators values decline was observed in 72 h after venom injection, which might indicate the beginning of regeneration of damaged heart tissue. However, further studies are needed to elucidate the precise mechanisms underlying the changes we found. Our findings may be useful for developing differentiated and more effective treatment strategies for the effects of these scorpion stings.

References

1. Jenkins TP, Ahmadi S, Bittenbinder MA, et al. Terrestrial venomous animals, the envenomings they cause, and treatment perspectives in the Middle East and North Africa. PLoS Negl Trop Dis 2021;15:e0009880.
2. Alqahtani AR, Badry A. Genetic diversity among different species of the genus *Leiurus* (Scorpiones: Buthidae) in Saudi Arabia and the Middle East. Saudi J Biol Sci 2020;27:3348-53.
3. Cid-Uribe JI, Veytia-Bucheli JI, Romero-Gutierrez T, et al. Scorpion venomics: a 2019 overview. Expert Rev Proteomics 2020;17:67-83.
4. Abroug F, Ouanes-Besbes L, Tilouche N, Elatrous S. Scorpion envenomation: state of the art. ICM 2020;46:401-10.
5. Yanchyshyn A. Features of morphometric parameters of the myocardium after rats' exposure to the scorpion *Leiurus macroctenus* venom. Dnipro State Medical University, «Morphologia» 2025;19:83-9.
6. Tobassum S, Tahir HM, Arshad M, et al. Nature and applications of scorpion venom: an overview. Toxin Rev 2020;39:214-25.
7. Zhang XY, Zhang PY. Scorpion venoms in gastric cancer. Oncol Lett 2020;12:196.
8. Gunas V, Maievskyi O, Synelnyk T, et al. Cytokines and their regulators in rat lung following scorpion envenomation. Toxicon X 2024;22:100198.
9. Reis MB, Zoccal KF, Gardinassi LG, Faccioli LH. Scorpion envenomation and inflammation: Beyond neurotoxic effects. Toxicon 2019;167:174-9.
10. Ahmadi S, Knerr JM, Argemi L, et al. Scorpion venom: detriments and benefits. Biomedicines 2020;8:18.
11. Gunas V, Maievskyi O, Raksha N, et al. The activity of metalloproteases and serine proteases in various organs after *Leiurus macroctenus* envenomation. J Toxicol 2023;5262729.
12. Cohen G, Burks SR, Frank JA. Chlorotoxin – a multimodal imaging platform for targeting glioma tumors. Toxins 2018;10:496.
13. Uzair B, Bint-e-Irshad S, Khan BA, et al. Scorpion venom peptides as a potential source for human drug candidates. Protein Pept Lett 2018;25:702-8.
14. Akef HM. Anticancer and antimicrobial activities of scorpion venoms and their peptides. Toxin Rev 2019;38:41-53.
15. Ozkan O, Filazi A. The determination of acute lethal dose-50 (LD50) levels of venom in mice, obtained by different methods from scorpions, *Androctonus crassicauda* (Oliver 1807). Turkiye Parazitol Derg 2004;28:50-3.
16. Yaqoob R, Tahir HM, Arshad M, Naseem S, Ahsan MM. Optimization of the conditions for maximum recovery of venom from scorpions by electrical stimulation. Pak J Zool 2016;48:265-69.
17. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
18. Crowther JR. The ELISA guidebook. 2nd ed. NJ: Humana Press; 2000.
19. Kaptein M, van den Heuvel E. Statistics for Data Scientists. Springer Cham; 2022.
20. Zoccal KF, Gardinassi LG, Bordon KCF, et al. EP80317 restrains inflammation and mortality caused by scorpion envenomation in mice. Front Pharmacol 2019;10:171
21. Roozbehani MP, Ghafourian M, Nemati M, et al. Evaluation of pro-inflammatory and anti-inflammatory cytokine levels in rats treated with *Hottentotta saulcyi* scorpion venom. Asia Pac J Med Toxicol 2023;11:152-6.
22. Resiere D, Mehdaoui H, Neviere R. Inflammation and oxidative stress in snakebite envenomation: a brief descriptive review and clinical implications. Toxins 2022;14:802.
23. Ziemkiewicz N, Hilliard G, Pullen NA, Garg K. The role of innate and adaptive immune cells in skeletal muscle regeneration. Int J Mol Sci 2021;22:3265.
24. Wang X, Zhou L. The many roles of macrophages in skeletal muscle injury and repair. Front Cell Dev Biol 2022;10:952249.
25. Cheng P, Li S, Chen H. Macrophages in lung injury, repair, and fibrosis. Cells 2021;10:436.
26. Strizova Z, Benesova I, Bartolini R, et al. M1/M2 macrophages and their overlaps – myth or reality? Clin Sci (Lond) 2023;137:1067-93.
27. Iacobazzi D, Convertini P, Todisco S, et al. New insights into NF-κB signaling in innate immunity: Focus on immunometabolic crosstalks. Biology (Basel) 2023;12:776.
28. Martin-Eauclaire MF, Adi-Bessalem S, Hammoudi-Triki D, et al. Serotherapy against voltage-gated sodium channel-targeting αtoxins from *Androctonus* scorpion venom. Toxins (Basel) 2019;11:63.
29. Zhang JY, Whalley JP, Knight JC, et al. SARS-CoV-2 infection induces a long-lived pro-inflammatory transcriptional profile. Genome Medicine 2023;15:69.
30. Feola A, Perrone MA, Piscopo A, et al. Autopsy findings in case of fatal scorpion sting: a systematic review of the literature. Healthcare (Basel) 2020;8:325.
31. Wan Q, Song D, Li H, He M-L. Stress proteins: the biological functions in virus infection, present and challenges for target-based antiviral drug development. Signal Transduct Target Ther 2020;5:125.
32. Matkivska R, Shchypanskyi S, Raksha N, et al. Cytokine disbalance in the rats' kidneys following *Leiurus macroctenus* envenomation. J Appl Biol Biotechnol 2024;12:110-5.
33. Fereidooni R, Shirzadi S, Ayatizadeh SH, et al. Scorpion envenomation-associated myocarditis: A systematic review. PLoS Negl Trop Dis 2023;17: 0011219.