ASIA PACIFIC JOURNAL of MEDICAL TOXICOLOGY
APJMT 14;2 http://apjmt.mums.ac.ir June 2025

ORIGINAL ARTICLE

The Effects of *Androctonus crassicauda* Scorpion Venom on Liver and Kidney Histopathology and Biochemical Factors in STZ-induced Diabetic Rats

FATEME JAFARI[1], MONA SHARIFIFARD[2], AMIR MOHAMAD AMIRI[3], NOOSHIN GHADIRI[3], MOHAMMAD NEMATI[4,*]

[1]Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Department of Vector Biology and Control of Disease, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[4]Department of Venomous Animals and Anti-venom Production, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Ahvaz, Iran

Abstract

Background: Type 2 diabetes (T2D) is a complex metabolic disorder characterized by insulin resistance and relative insulin deficiency, leading to chronic hyperglycemia. The potential therapeutic applications of scorpion venom have gained attention in recent years, particularly in the context of diabetes management. Therefore, our aim is to investigate the effect of *A. crassicauda* scorpion venom on biochemical factors of streptozotocin (STZ)-induced diabetic rats.
Methods: In this study, diabetes was defined as any blood sugar level over 250 mg/dL in male Wistar rats induced by a single dose of streptozotocin intraperitoneally. The animals were divided into five groups, two of which received scorpion venom. Afterwards, the levels of blood glucose, cholesterol, triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), urea, creatinine, alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate aminotransferase (AST) were measured. In addition, histopathological changes in the liver and kidneys were assessed using hematoxylin and eosin (H&E) staining.
Results: It was found that *Androctonus crassicauda* scorpion venom reduced blood sugar. Other biochemical factors were also decreased compared to the diabetic group, and histological analysis showed partial repair of degenerated liver and kidney cells after venom injection compared with diabetic rats not treated with venom.
Conclusion: The results of this study indicate that the effect of *Androctonus crassicauda* scorpion venom on diabetes is positive and reduces diabetic symptoms. Therefore, scorpion venom may be a viable option for managing diabetes in the future.

Keywords: *Androctonus crassicauda*, Scorpion venom, Type 2 diabetes (T2D)

How to cite this article: Jafari F, Sharififard M, Amiri A, Ghadiri N, Nemati M. The Effects of Androctonus crassicauda Scorpion Venom on Liver and Kidney Histopathology and Biochemical Factors in STZ-induced Diabetic Rats. Asia Pac J Med Toxicol 2025; 14(2): 41- 46.

INTRODUCTION

Globally, diabetes is a major public health concern, as the incidence and prevalence of both type 1 and type 2 diabetes are on the rise [1]. Approximately 442 million people are currently affected by diabetes, a significant increase from 108 million in 1980 [2]. The majority of diabetes cases worldwide are caused by type 2 diabetes, which results from a progressive defect in insulin secretion and/or insulin resistance [3]. The growing prevalence of type 2 diabetes poses a major public health challenge, particularly in low- and middle-income countries [4]. The increasing trend of type 1 diabetes, which is characterized by an absolute insulin deficiency, has primarily been observed in countries with high incomes, such as the United States and Europe [1]. There is also a significant concern with prediabetes, a condition of impaired glucose regulation that precedes the development of overt diabetes [5]. The risk of developing type 2 diabetes and related complications is high among individuals with prediabetes, including cardiovascular disease and chronic kidney disease [6]. According to epidemiological studies, one-third of adults with newly diagnosed diabetes already have kidney damage, which implies that hyperglycemia may contribute to the development of complications even before glucose levels reach the threshold for diagnosis [7].

Various pathophysiological mechanisms contribute to diabetes and its complications, including oxidative stress, inflammation, endoplasmic reticulum stress, aberrant insulin signaling, and altered substrate metabolism and mitochondrial bioenergetics [8]. Microvascular complications, such as diabetic nephropathy and retinopathy, as well as macrovascular complications, such as cardiovascular disease, are associated with these pathways [9].

*Correspondence to: Dr. Mohammad Nemati. Department of Venomous Animals and Anti-venom Production, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Ahvaz, Iran.
E-mail: dr_mnemati58@yahoo.com. Tel: +98-9166614134

Several potential therapeutic approaches are currently being explored in the quest for a cure for diabetes. Pharmaceutical interventions, such as hypoglycemic agents and medications targeting specific pathways involved in diabetic complications, have shown promising results [10]. Scorpion venom is one of these biological agents.

The venom of a scorpion contains a wide variety of peptides, proteins, enzymes, and other molecules [11]. Moreover, scorpion venom has been demonstrated to possess antimicrobial, antiviral, and anticancer properties [12]. Various compounds in scorpion venom can affect glucose metabolism and insulin secretion, resulting in hypoglycemia. While it is not fully understood how scorpion venom works, it is believed that it can increase glucagon, cortisol, and thyroid hormone levels, modulate thyroid hormone activity, or influence insulin secretion [13].

It has been established that the venom of scorpions has antidiabetic effects in various animal models of diabetes [14, 15]. Several studies have demonstrated that scorpion venom or its components may reduce blood glucose levels and improve other metabolic parameters in diabetic animals [14, 16, 17]. It has been shown, for instance, that the venom of the scorpion *Scorpio maurus palmatus* is capable of reducing the development of diabetes in mice induced with alloxan [14]. As a result of its antidiabetic properties, scorpion venom may stimulate the secretion of glucagon, while inhibiting the release of insulin [16].

There is evidence to suggest that scorpion venom and its bioactive components have promising antidiabetic properties, making them candidates for development as novel therapeutic agents for diabetes and associated metabolic disorders. As a result, the purpose of this study is to investigate the effects of Androctonus *crassicauda* scorpion venom on blood sugar levels and biochemical parameters in rats with streptozotocin-induced diabetes.

METHODS

Animals and Scorpions

A total of 30 male Wistar-Albino rats aged 8–10 weeks and weighing between 200–250 g were purchased from Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran. The animal maintenance center maintained a 12-hour light/dark cycle and provided standard water and food to all animals. Scorpions of the species *A. crassicauda* were collected in Khuzestan Province, Iran, and kept in Iran's Razi Vaccine and Serum Research Institute (Ahvaz). In addition to providing water *ad libitum*, the scorpions were also provided with cockroaches on a weekly basis. This research was conducted in accordance with the guidelines of the Laboratory Animal Ethics Committee of AJUMS (Ethics code: IR.AJUMS.AEC.1403.046).

Venom Collection and Lethal Dose Assessment

As explained by Ozkan and Filazi [18], scorpion venom is extracted by applying low-voltage electrical stimulation. The scorpion was immersed in saline solution to enhance electrical conduction and shocked using an electrode. The venom droplet was collected in a Petri dish. A 12-volt battery served as the power source. The venom was stored at –80 °C. A bicinchoninic acid (BCA) protein assay kit was used to measure venom protein concentration (Parstous Biotechnology, Iran). The lethal dose (LD_{50}) was calculated using the Spearman-Karber method in NMRI mice and converted to a weight-based LD_{50} for Wistar rats [19].

Induction of Diabetes and Experimental Design

A description of the induction protocol is provided by Furman [20]. Briefly, a minimum of 8 hours of fasting was required prior to induction. A single intraperitoneal (i.p.) injection of streptozotocin (STZ, Solarbio, China) (55 mg/kg), dissolved in 0.1 M cold citrate buffer (pH 4.5), was used. Blood glucose concentrations were monitored every two days using a glucometer (Iran). Rats with hyperglycemia (>250 mg/dL) were considered diabetic. Animals were randomly divided into five groups (n=6):

- Control group: received normal saline (i.p.).
- Group I: received STZ (55 mg/kg).
- Group II: received STZ (55 mg/kg) and metformin (500 mg/kg).
- Group III: received STZ (55 mg/kg) and scorpion venom (0.1 mg/kg, LD_{25}).
- Group IV: received STZ (55 mg/kg) and scorpion venom (0.2 mg/kg, LD_{50}).

Venom and metformin injections were administered weekly for three weeks (days 10, 17, and 24). On day 30, animals were anesthetized using ketamine (90 mg/kg) and xylazine (10 mg/kg), and blood was collected via cardiac puncture into gel tubes. Serum was separated and stored at –20 °C until analysis. Liver and kidney tissues were rinsed with saline and fixed in 10% formalin for histological examination.

Assay of Biochemical Factors

Biochemical markers (cholesterol, triglycerides (TG), HDL, LDL, urea, creatinine, ALP, ALT, AST) were measured. Blood was collected in tubes without anticoagulant, centrifuged at 3000 rpm for 10 minutes, and serum was isolated. A Hitachi 912 auto-analyzer (Japan) and an enzymatic colorimetric kit (Pars Azmoon, Iran) were used.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum was obtained by centrifuging whole blood at 3000 rpm for 10 minutes. Insulin concentrations were measured using ELISA kits (Sunlong, China) according to the manufacturer's instructions.

Histopathological Examinations

Liver and kidney samples were fixed in 10% formalin for three days. After dehydration, tissues were embedded in paraffin and sectioned using a microtome. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (DIALUX 20 EB) at 40× magnification. Identical settings were used for all images.

Statistical Analysis

GraphPad Prism version 10 (GraphPad, USA) was used for statistical analysis and graphing. Data are presented as mean ± standard deviation (SD). Normality was tested using the Shapiro–Wilk test. Two-way ANOVA followed by Tukey's multiple comparison test was performed. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of *A. crassicauda* Venom on Blood Glucose and Insulin

In this study, diabetic rats induced with STZ (Group I)

showed an increase in blood glucose levels compared with normal controls, while diabetic rats receiving metformin (Group II) showed a decrease in blood glucose levels. However, both concentrations (Group III, Group IV) of *A. crassicauda* venom decreased blood glucose levels in comparison to Group I rats. However, this decrease was not statistically significant compared to Group II rats (Figure 1A).

Effect of *A. crassicauda* on Serum Lipids

Compared with control rats, diabetic rats of Group I showed significantly increased levels of cholesterol, TG, HDL, and LDL plasma lipids, whereas diabetic rats of Group II showed significantly reduced levels of these factors. In groups that received venom, all of the lipid factors were decreased, but only TG level reductions were significant. While all factors were lower in Group IV rats compared to Group I rats, only TG and LDL decreased significantly. The Group III and Group IV groups did not differ significantly (Figure 2).

Effect of *A. crassicauda* on Kidney Biochemical Factors

There was a significant increase in biochemical factors associated with kidney function, including urea and creatinine, in Group I rats compared to the control group, whereas Group II rats showed a significant decrease in these factors. Furthermore, in the groups that received venom (Group III and Group IV), the reduction was significant compared to Group I. However, there was no significant difference between Group III and Group IV rats (Figure 3).

Effect of *A. crassicauda* on Liver Biochemical Factors

Group I rats displayed a substantial increase in

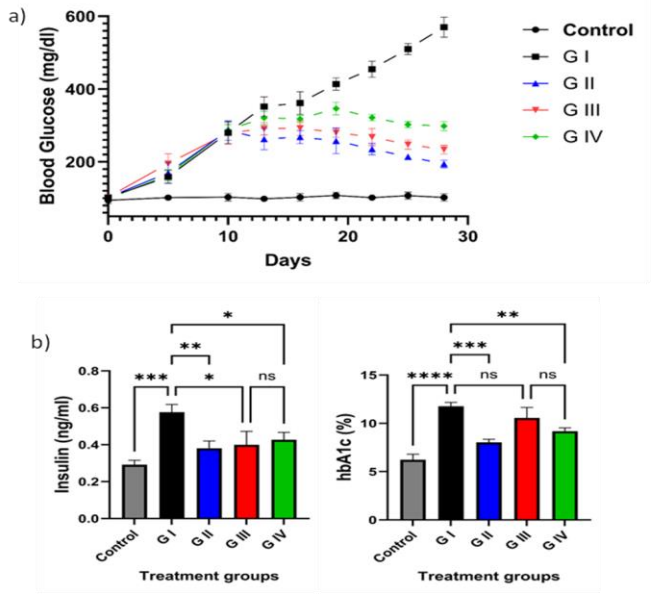

Figure 1. a) *A. crassicauda* venom caused a decrease in blood glucose levels. b) shows the insulin and hbA1C levels. GI is STZ-induced diabetic rats (black), G II is STZ-induced diabetic rats that received metformin (blue), G III is STZ-induced diabetic rats that received scorpion venom with 0.1 mg/Kg (red), G III is STZ-induced diabetic rats that received scorpion venom with 0.2 mg/Kg (green), Data presented are mean ± SD (n=6). * p<0.05, ** p<0.01, p<0.001, p<0.0001 and ns is non-significant

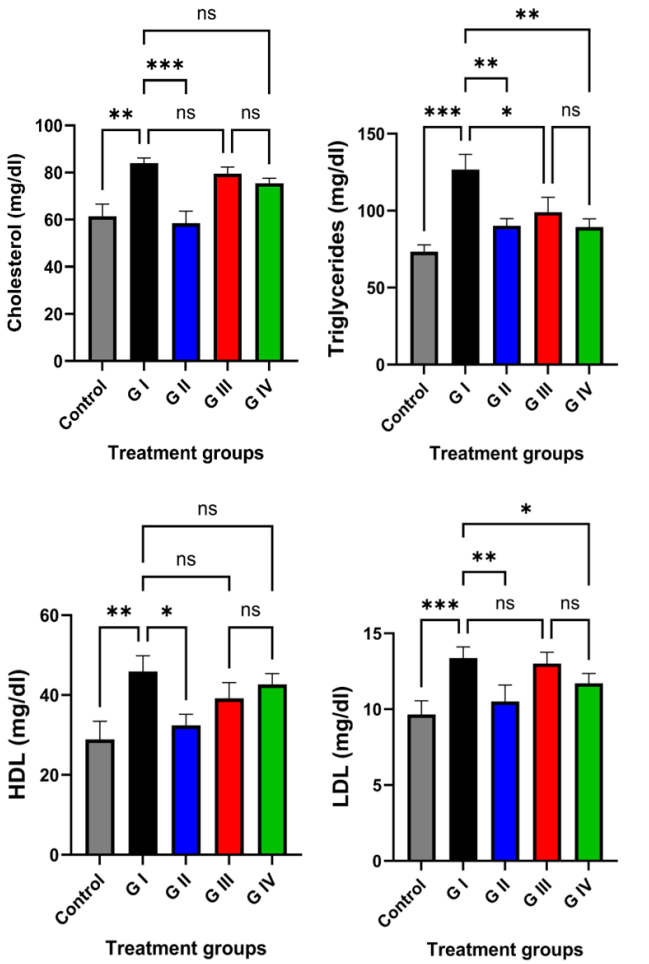

Figure 2. *A. crassicauda* venom effect on serum lipids G I is STZ-induced diabetic rats (black), G II is STZ-induced diabetic rats that received metformin(blue), G III is STZ-induced diabetic rats that received scorpion venom with 0.1 mg/Kg (red), G III is STZ-induced diabetic rats that received scorpion venom with 0.2 mg/Kg (green), Data presented are mean ± SD (n=6). * p < 0.05, ** p < 0.01, *** p < 0.001, **** p< 0.0001 and ns is non-significant

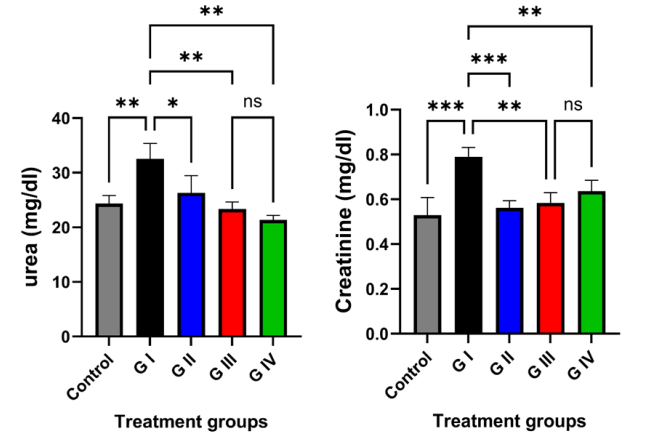

Figure 3. *A. crassicauda* venom effect on kidney biochemical factors. G I is STZ- induced diabetic rats (black), G II is STZ-induced diabetic rats that received metformin(blue), G III is STZ-induced diabetic rats that received scorpion venom with 0.1 mg/Kg (red), G III is STZ-induced diabetic rats that received scorpion venom with 0.2 mg/Kg (green), Data presented are mean ± SD (n=6). * p < 0.05, ** p < 0.01, *** p < 0.001, **** p< 0.0001 and ns is non-significant.

biochemical factors associated with liver function, such as ALT, ALP, and AST, when compared to the control group. Group II rats had a marked decrease in these biochemical factors compared to Group I rats. A significant reduction was also observed in the groups that received venom (Group III and Group IV) compared to Group I rats. However, no significant difference was observed between Group III and Group IV rats (Figure 4).

Effect of *A. crassicauda* on Liver and Kidney Histopathology

An examination of the prepared sections revealed necrosis in some liver hepatocytes of the Group I rats. In addition, a large number of hepatocytes showed signs of degeneration. Cellular swelling and fatty changes were evident from the empty cytoplasm of these cells. In the venom treatment groups (Group III and Group IV), these pathological changes were reduced. Cellular swelling and fatty changes were observed in a small number of hepatocytes. There was also a reduction in the number of cells that had undergone degeneration in the Group II rats. In the control group, the liver showed a normal structure (Figure 5).

Upon microscopic examination of kidney sections, Group I rats were found to have necrosis and degeneration of renal tubular epithelial cells. In these cells, many empty spaces were observed, and some of the cells had become necrotic. There was a reduction in the number of cases and the number of damaged tubules in the venom treatment groups (Group III and Group IV). It was also found that there was a reduction in the number of tubules with degenerated lining cells in the Group II rats. A normal kidney structure was observed in the control group (Figure 6).

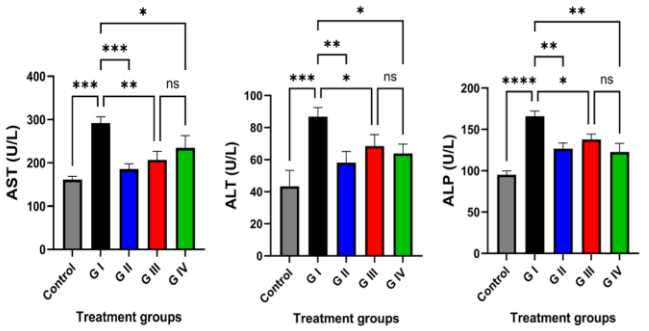

Figure 4. *A. crassicauda* venom effect on liver biochemical factors. G I is STZ- induced diabetic rats (black), G II is STZ-induced diabetic rats that received metformin(blue), G III is STZ-induced diabetic rats that received scorpion venom with 0.1 mg/Kg (red), G III is STZ-induced diabetic rats that received scorpion venom with 0.2 mg/Kg (green), Data presented are mean ± SD (n=6). * p < 0.05, ** p < 0.01, *** p < 0.001, **** p< 0.0001 and ns is non-significant.

DISCUSSION

The prevalence of diabetes in the population makes it necessary to find new drugs for managing it, and one of the most neglected therapies is that derived from natural sources, such as venoms [10, 21]. It has been possible to develop new drugs and treatments through the use of venoms [22, 23]. One such example is *A.* crassicauda scorpion venom, and this study investigated the effects of different doses of *A. crassicauda* venom on serum biochemical factors in STZ-induced diabetes in rats. The results of our study indicate that the venom of the *A. crassicauda* scorpion can lower blood glucose levels at both concentrations, and this blood glucose reduction is accompanied by a decline in hyperinsulinemia caused by type 2 diabetes, suggesting that this venom may be a potential new target for managing diabetes-related

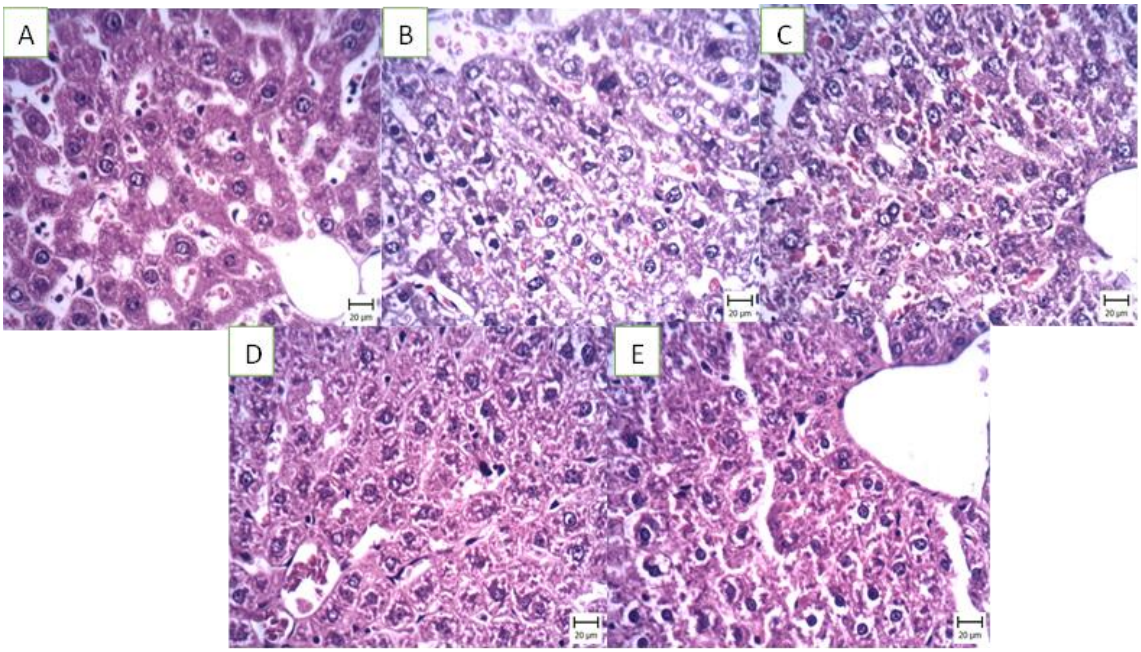

Figure 5. Liver histopathology. A is control group, B is G I (STZ- induced diabetic rats), C is G II (STZ-induced diabetic rats that received metformin), D is G III (STZ-induced diabetic rats that received scorpion venom with 0.1 mg/Kg), E is G IV (STZ-induced diabetic rats that received scorpion venom with 0.2 mg/Kg).

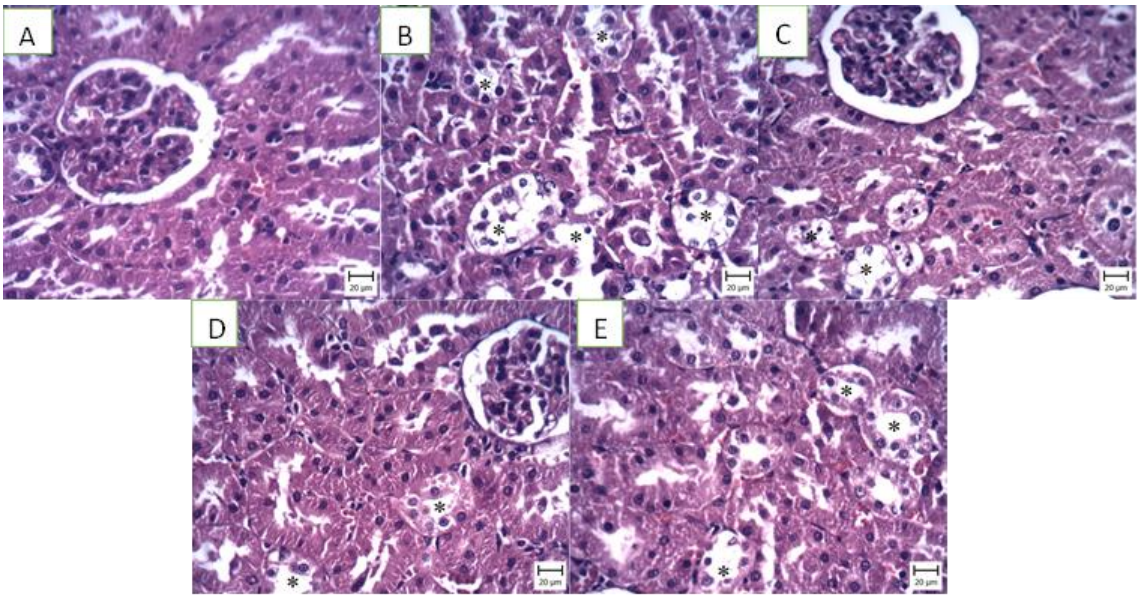

Figure 6. Liver histopathology. A is control group, B is G I (STZ- induced diabetic rats), C is G II (STZ-induced diabetic rats that received metformin), D is G III (STZ-induced diabetic rats that received scorpion venom with 0.1 mg/Kg), E is G IV (STZ-induced diabetic rats that received scorpion venom with 0.2 mg/Kg).

symptoms. Furthermore, the animals that had received venom showed a decrease in TG levels, which is one of the hallmarks of diabetes. Additionally, the venom demonstrated a potential to improve the function of both the kidneys and liver, as reflected by a significant reduction in serum biochemical factors related to both organs when compared with diabetic rats. Furthermore, this improved functionality is demonstrated by histological examination of these organs, which showed less degeneration and necrosis caused by diabetes as compared to diabetic control groups. However, no significant difference was observed between the two venom doses.

The results of our study are in agreement with those of Roudbari et al., who reported that the venom of *A. crassicauda* scorpion may reduce blood glucose levels in diabetic rats [24]. Our findings provide more detailed data on serum biochemical parameters.

Moreover, Shahdadi et al. reported that snake venom from the species *Naja naja oxiana* can be used as an antidiabetic agent due to its ability to reduce blood glucose levels and decrease TG levels in rats [25]. The results of the study support our conclusion that venoms, as biologically active agents, may be effective in managing diabetic symptoms.

Xie et al. conducted another study in which scorpion body extract was combined with gypsum to reduce blood sugar levels and serum lipid levels in diabetic mice. Their study did not focus on the scorpion venom [16]. However, their findings confirmed our hypothesis that scorpions can have an antidiabetic effect and may serve as a good target for developing new drugs to treat diabetic symptoms.

Kumar et al. determined that *Aloe* extract can result in a significant reduction of blood glucose and total cholesterol [26]. This finding is in agreement with ours and emphasizes the importance of using biological agents for managing the more severe symptoms of diabetes.

The overall conclusion of our study suggests that the venom of *A. crassicauda* scorpion venom causes a decrease in glucose levels in diabetic rats and facilitates improved function of the liver and kidneys as a whole.

CONCLUSION

Scorpion venom from *A. crassicauda* causes hypoglycemia in diabetic rats and combats hyperinsulinemia caused by type 2 diabetes. Additionally, the assessment of serum biochemical factors demonstrated that scorpion venom could be an effective drug discovery target against diabetes, as it improves glycemic control and enhances kidney and liver function in diabetic conditions.

ACKNOWLEDGMENT

The authors express their gratitude to the Razi Vaccine and Serum Research Institute.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding and Support: This study was funded by Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, (Grant No. O3S70).

Ethical Approval: This study was conducted under the supervision of the Biomedical Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Approval ID: IR.AJUMS.AEC.1403.046).

REFERENCES

1. Lin X, Xu Y, Pan X, Xu J, Ding Y, Sun X, et al. Global, Regional, and National Burden and Trend of Diabetes in 195 Countries and Territories: An Analysis From 1990 to 2025. Sci Rep. 2020;10(1).
2. Mohamed SF, Mwangi M, Mutua MK, Kibachio J, Hussein A,

Ndegwa Z, et al. Prevalence and Factors Associated With Pre-Diabetes and Diabetes Mellitus in Kenya: Results From a National Survey. BMC Public Health. 2018;18(S3).
3. Nazir MA, AlGhamdi L, Alkadi M, AlBeajan N, AlRashoudi L, AlHussan M. The Burden of Diabetes, Its Oral Complications and Their Prevention and Management. Open Access Maced J Med Sci. 2018;6(8):1545-53.
4. Khan MA, Hashim M, King J, Govender RD, Mustafa H, Kaabi JA. Epidemiology of Type 2 Diabetes – Global Burden of Disease and Forecasted Trends. J Epidemiol Glob Health. 2019;10(1):107.
5. Hostalek U. Global Epidemiology of Prediabetes - Present and Future Perspectives. Clin Diabetes Endocrinol 2019;5(1).
6. Huang Y, Cai X, Mai W, Li M, Hu Y. Association Between Prediabetes and Risk of Cardiovascular Disease and All Cause Mortality: Systematic Review and Meta-Analysis. BMJ. 2016:i5953.
7. Echouffo-Tcheugui JB, Narayan KMV, Weisman D, Golden SH, Jaar BG. Association Between Prediabetes and Risk of Chronic Kidney Disease: A Systematic Review and Meta-analysis. Diabet Med. 2016;33(12):1615-24.
8. Kenny H, Abel ED. Heart Failure in Type 2 Diabetes Mellitus. Circ Res. 2019;124(1):121-41.
9. Fan W. Epidemiology in Diabetes Mellitus and Cardiovascular Disease. Cardiovasc Endocrinol. 2017;6(1):8-16.
10. Brannick B, Dagogo-Jack S. Prediabetes and Cardiovascular Disease. Endocrinol Metab Clin North Am. 2018;47(1):33-50.
11. Zeng XC, Li W, Wang SX, Zhu S, Luo F. Precursor of a Novel Scorpion Venom Peptide (BmKn1) With No Disulfide Bridge From Buthus Martensii Karsch. Iubmb Life. 2001;51(2):117-20.
12. Hong W, Li T, Song Y, Zhang R, Zeng Z, Han S, et al. Inhibitory Activity and Mechanism of Two Scorpion Venom Peptides Against Herpes Simplex Virus Type 1. Antiviral Res. 2014;102:1-10.
13. Oliveira GHd, Cerni FA, Cardoso IA, Arantes EC, Pucca MB. Tityus Serrulatus Envenoming in Non-Obese Diabetic Mice: A Risk Factor for Severity. J Venom Anim Toxins Incl Trop Dis. 2016;22(1).
14. Abdel-Rahman MA, Mohammed AH, Ahmed SH, Binnaser YS, Abdel-Nabi IM. Antidiabetic Effect of the ScorpionScorpio Maurus Palmatus</I>body Extract Using Alloxan-Induced Diabetic Mice Model. J Taibah Univ Sci. 2019;13(1):504-13.
15. Ortíz E, Gurrola GB, Schwartz EF, Possani LD. Scorpion Venom Components as Potential Candidates for Drug Development. Toxicon. 2015;93:125-35.
16. Xie W, Zhao Y, Gu D, Du L, Cai G, Zhang Y. Scorpion in Combination With Gypsum: Novel Antidiabetic Activities in Streptozotocin-Induced Diabetic Mice by Up-Regulating Pancreatic PPARγ and PDX-1 Expressions. Evid Based Complement Alternat Med. 2011;2011(1).
17. Farrag ARH, Khaled HA, Tewfick MK, Kamel JAH. The Effects of Androctonus Amoreuxi Scorpion Extract and Sitagliptin in the Treatment of Diabetes Mellitus Type 2 in Animal Models. Egypt Acad J Biol Sci B Zool. 2016;8(1):75-86.
18. 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. 1807:50-3.
19. Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. World J Virol. 2016;5(2):85-6.
20. Furman BL. Streptozotocin-Induced Diabetic Models in Mice and Rats. Curr Protoc. 2021;1(4):e78.
21. Ghadiri N, Javidan M, Sheikhi S, Taştan Ö, Parodi A, Liao Z, et al. Bioactive peptides: an alternative therapeutic approach for cancer management. Front Immunol. 2024;15:1310443.
22. Koh DC, Armugam A, Jeyaseelan K. Snake venom components and their applications in biomedicine. Cell Mol Life Sci. 2006;63(24):3030-41.
23. Harvey AL. Toxins and drug discovery. Toxicon. 2014;92:193-200.
24. L. Roudbari SI. The effects of Anderoctonus Crassicauda scorpion venom in the treatment of Diabetes Mellitus type 1 in animal models. Ann Biol Res. 2012;3(12):5782-5.
25. Shahdadi S, Hamidi F, Fathi B. The effect of Iranian snake, Naja naja oxiana venom on the blood glucose concentration and some biochemical parameters of experimental diabetic rats. Heliyon. 2024;10(2):e24436.
26. Kumar R, Sharma B, Tomar NR, Roy P, Gupta AK, Kumar A. In vivo evaluation of hypoglycemic activity of Aloe spp. and identification of its mode of action on GLUT-4 gene expression in vitro. Appl Biochem Biotechnol. 2011;164(8):1246-56.