Antidiabetic Effect of Scorpion Venom (*Hottentota tamulus* and *Androctonus finitimus*) Using Alloxan Induced Diabetic Mice Model

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Abstract

The antidiabetic effect of venom of *Hottentota tamulus* and *Androctonus finitimus* scorpions was evaluated in the alloxan-induced diabetic mice by studying biological parameters and pancreas structure abnormalities. Diabetes is a metabolic disorder characterized by elevated blood glucose levels due to either insufficient insulin production (Type 1) or impaired insulin function (Type 2), leading to various health complications. For extraction of venom, 50 scorpions of *Hottentota tamulus* and *Androctonus finitimus* species were maintained in the lab. Venom of both species was characterized and its effect was evaluated on diabetes. In this study, 32 male albino mice were divided into eight main groups each of 4 mice. Group 1 was injected intraperitoneally with physiological saline solution, Group 2 was given alloxan but no treatment was given. Group 3 received crude venom (150 mg/kg) (i p., daily for 5 weeks) after induction of alloxan. Group 4 received crude venom (300 mg/kg). Group 5 received venom fraction I (1.3mg/kg), Group 6 was given venom fraction II (1.4mg/kg), Group 7 was given venom fraction III (1.5mg/kg) and group 8 received recommended dose (1.8mg/kg) of metformin. This grouping of mice was repeated for *Hottentota tamulus* and *Androctonus finitimus* scorpions. Blood samples were collected from all groups to check effect of crude venom and venom fractions on fasting blood glucose level, insulin level, lipid profile level and body weight in control group and treated groups. Administration of venom revealed a significant decrease (P<0.0001) in the concentrations of glucose, body weight was gradually significantly increased (P<0.0001) in venom treated groups and metformin treated groups. Venom treatments also showed a significant elevation in triacylglyceride (P<0.0001), total cholesterol, LDL-C and a significant reduction (P<0.0001) in HDL-C and plasma insulin levels.

Introduction

Diabetes mellitus (DM) is a collection of metabolic illnesses in which mild hyperglycemia is brought on by inadequate insulin synthesis, insufficient insulin action, or both. As a result, there are carbohydrate, lipid, and protein shortages [1]. Diabetes may be classified into two basic categories: type 1 and type 2 diabetes [2]. Across the globe 90% of individuals with diabetes have type 2 diabetes mellitus, which has affected 463 million people worldwide. It is a serious condition that may be managed with both insulin and non-insulin medications. In case of non-insulin treatment, several drugs, such as metformin, sulfonylureas, sodium-glucose cotransporter 2 inhibitors, and glucagon-like peptide 1 receptor inhibitors, are taken orally [3].

Several other approaches of managing DM include exercising, maintaining a healthy diet, taking common chemical hypoglycemic drugs (such as sulfonylureas, biguanides, and meglitinide analogues), administering insulin, and using INGAP (islet neogenesis associated protein) to boost pancreatic islet survival and cell regeneration. In vitro studies on mice models have proven that extracts from a variety of medicinal plants also have potent anti-diabetic effects [4,5]. In previous studies Xie and colleagues reported that scorpion extract (Buthus martensii kirsch) combined with gypsum revealed a novel antidiabetic activity in diabetic mice (induced by streptozotocin) through upregulating the expression of pancreatic PPARγ (peroxisome proliferator-activated receptor gamma) and PDX-1 (pancreatic and duodenal homeobox 1), improving islet regeneration and enhancing insulin secretion. The antidiabetic effect and mode of action of Socrpio maurus palmatus body extract in diabetic mice induced by alloxan evaluated that the administration of scorpion extract significantly elevated the level of plasma insulin which was associated with a remarkable decrease in the level of blood glucose in diabetic mice. Interestingly, scorpion extract significantly increased the number of β-cells and the size of pancreatic islets in diabetic mice [6].

In Pakistan there are five species of scorpion *Hottentota tamulus* and only one specie of *Androctonus finitimus*. These two species are used in this research to check different biological parameters of alloxan induced mice using scorpion venom. The main objective of this study is to investigate the antidiabetic effect of scorpions (*Hotentota tamulus* and *Androctonus finitimus*) in alloxan induced diabetic mice model.

Materials and Methods

Collection of mice

The present investigation was employed on male albino mice weighing between 18 and 25 g. The animals were preserved in polyethylene cages and fed on standard laboratory food with water ad libitum. They fasted for 8h before performing the experiments.

Collection of scorpions and their maintenance

Collection of scorpions was conducted from February 2023 to May 2023. Two common scorpions *Hottentota tamulus* (n=60) (Figure 1) and *Androctonus finitimus* (n=60) (3.2 B) belonging to Buthidae family were collected from sandy areas of Mianwali and Sargodha Districts of Punjab, Pakistan (i.e., Mianwali: Chak # 16/DB, 32°38'77" N, 71°31'69" E; Bhagtanwala: Chak # 34/SB, 31°55'59" N, 72°54'22" E; Chak # 76/SB,

32°06'74" N, 72°52'38" E; Chak # 24/ SB, 32°02'07" N, 72°55'48" E).

Electrical extraction of venom

A method used for extraction of venom was an electrical stimulation of telson [7]. The scorpion was placed on a petri dish with adhesive tape for venom extraction. Electric current (25 V) was administered at the base of the telson for 5 seconds with the use of a pointed electrode to shock the scorpions until the venom was expelled (Figure 2). The scorpion body was submerged in a 10% NaCl solution for greater electrical current conductivity. In a graduated capillary tube, the venom was collected and combined with distilled water in a 1:2 ratio (venom:distilled water). To remove insoluble venom particles, diluted venom was centrifuged for 10 minutes at 10000xg in a centrifuge (MPW-352R). The supernatant was collected and lyophilized in the falcon tube. The Bradford technique was used to determine the protein content of

Figure 1: Dorsal view of scorpions (A) *Hottentota tamulus* (B) *Androctonus finiimus*.

Figure 2: Methods of venom extraction (Electrical Method).

both species' venom.

Protein content measurement

In accordance with manufacturer's instructions, protein content of crude venom and fractions was assessed using the Bio-Rad Quick start Bradford protein assay kit.

Characterization of venom

Venom was characterized through two different ways:

SDS-PAGE

SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) was used to examine the protein components of the crude venom, allowing the researchers to observe both low and high molecular weight peptides in the same gel.

Fractionation of venom by HPLC

The venom was separated using high performance liquid chromatography (HPLC). The crude venom (6 mg) was dissolved in 0.05% trifluoroacetic acid in HPLC grade water. After that, it was centrifuged for 15 minutes at 15000xg. Purification was carried out using clear supernatant. Reverse phase HPLC was carried out in an HPLC system (LC 20T; Shimadzu, Japan) at flow rate of 1 ml/min for 180 minutes through C 18 column (250 mm x 4.6 mm) with a gradient of trifluoroacetic acid in water (0.1%) (Solution A) and acetonitrile (Solution B) 5% for 10 min, followed by 5-15% for 20 min, 15-45% for 120 min and 45-78% for 20 min. Protein fractions were detected at 214 nm, manually collected and dried in a concentrator, and kept at -20°C. To construct a venom code for species determination the most reproducible peaks from HPLC chromatograms were selected for MALDI analysis.

Mass spectroscopy

MALDI-TOF MS (matrix-assisted laser desorption ionisation time-of-flight mass spectrometry) was used to analyse crude venom and fractions from both species on a MALDI-TOF/TOF Autoflex III (Bruker Daltonik GmbH). The sample was prepared using the dried droplet technique. A sample (2 l) was combined with an 8 lul solution of α-cyanohydroxycinnamic acid (10-12 mg/ml in 0.1% TFA in acetonitrile and 0.1% TFA in water, in a 1:2 ratio) described how 1ul of material was spotted on a MALDI plate (MTP Anchor chip var. 384), dried, and analysed in linear positive mode.

Induction of diabetes in mice using alloxan

Diabetes was induced in mice using alloxan. Mice (18-25 g in weight) was undergone a 24-hour fast while having unrestricted access to water in order to induce diabetes in them. To induce alloxan in fasting animals, freshly made solutions was used. After 72 hours the alloxan injection, the blood sugar levels was checked. The present investigation about diabetic mice, defined as those with a persistently increased blood glucose level (>200 mg/dL) [8].

Experimental approach

Thirty two male albino mice were taken for experimental plan. Diabetes was induced in the mice by giving alloxan. In this study, 20 male albino mice (18-25 g) were divided into five main groups each of 4 mice. Group 1 was negative control group and it was injected with physiological saline solution (0.9% NaCl), Group 2 was positive control group as it was given alloxan but no treatment was given. Group 3 received crude venom (150 mg/kg) after induction of alloxan. Group 4 received crude venom (300 mg/kg). Group 5 received venom fraction I (1.3mg/kg), Group 6 was given venom fraction II (1.4mg/kg), Group 7 was given venom fraction III (1.5mg/kg) and group 8 received recommended dose of metformin. This grouping of mice was repeated for *Hottentota tamulus* and *Androctonus finitimus* scorpions. Blood samples were collected from all groups to check effect of crude venom and venom fractions on fasting blood glucose level, insulin level, lipid profile level and body weight in control group and treated groups.

Histopathological examination

Four mice from each group were selected to histopathological study. The pancreas was isolated from the animals after sacrificing and rinsed in ice-cold saline and fixed in 10% formaldehyde overnight for a maximum of 12 h. The tissues were dehydrated and cleared before being embedded in paraffin wax. The pancreas was cut into three sections: posterior, middle and the anterior parts. Paraffin sections were segmented into 5-micron thickness and fixed on to glass slide. They were deparaffinized in xylene twice for 5 min and then rehydrated with graded alcohol and stained with hematoxylin and counterstained with eosin (H&E) dye. Colorimetric commercial kits were used to assess several biochemical parameters such as glucose level, insulin level, weight, cholesterol level, and Triacylglycerol, LDL-C and HDL-C. LDL-C showed a significant elevation, while HDL-C levels represented a significant reduction in the positive control group compared to the negative control group ($P<0.0001$).

Statistical analyses

Data were statistically studied using SPSS software. For all measurements in control and treated groups, descriptive analyses (Mean ± SEM) were applied. The potential effect of scorpion venom and venom fractions on healthy and diabetic mice was checked by one-way ANOVA test followed by Tukey's post hoc test. Differences between control group and treated group were considered statistically significant at $P < 0.05$.

Results and Discussions

Characterization of electrically extracted Venom

SDS-PAGE: The peptides bands of venom from the scorpion species were also compared. Table 1 shows that peptides of molecular weight ≥17 were found in the venom of both species. Most prominent and intense band which molecular is above 180kDa was separated in the *A. finitimus* but not observed in the *H. tumulus*. High molecular weight proteins were characterized using SDS-PAGE; however peptides with molecular weight less than 10kDa were not characterized so venom was further analyzed through HPLC and mass spectrometry.

Comparison of protein bands deducted in electrophoretic analysis of venom from different Scorpion species (Figure 3).

HPLC: Venom of *H. tamulus* and *A. finitimus* extracted by electrical method was fractionated via HPLC. Further analysis of these fractions was carried out by MALDI-TOF M.

Approximately 6 mg crude venom was fractionated on C 18 column by reverse phase HPLC at the flow rate of 1 ml/min for 180 minutes. Fractions were collected and lyophilized. Major fractions are labeled and further analyzed by MALDI-TOF MS.

Effect of scorpion venom on body weight: Tables 2 and 3 summarize the

Table 1: Mass fingerprint of venom peptides of different fractions of *H. tamulus* by MALDI analysis.

Fractions	RT (min)	Molecular masses (m/z)				Identified Toxins		[M + H]+ Refrences
Fraction I	8.75	666.1	877.1					
Fraction II	35.8	1596.2	3190.5			α KTx 20.1	3190	Abdel-Mottaleb et al., 2006
Fraction III	48.5	877.0	1633.1	3264.3	3573.3	α-kTx 8.1	3473.4	Pimenta et al., 2001
Fraction IV	56.6	951.5	2947.5					
Fraction V	72.4	3110.6	3554.7	3727.5	3932.6	TsKα[31]	3779.4	Pimenta et al., 2001 Dhawan et al., 2002
						BtITx3	3796	
Fraction VI	77.8	814.6	2966.3					
Fraction VII	81.8	1661.9	3507.9	3525.0	3560.5	α-kTx 15.5	3781.1	Dhawan et al., 2002
Fraction VIII	88.9	3390.8						
Fraction IX	102.4	2687.6						
Fraction X	112.0	3345.0	6688.5	6911.3	7036.2	TsNTxPNH2	6694.6	Pimenta et al., 2001

Figure 3: Electrophoretic separation of scorpion venom peptides on SDS polyacrylamide gel.

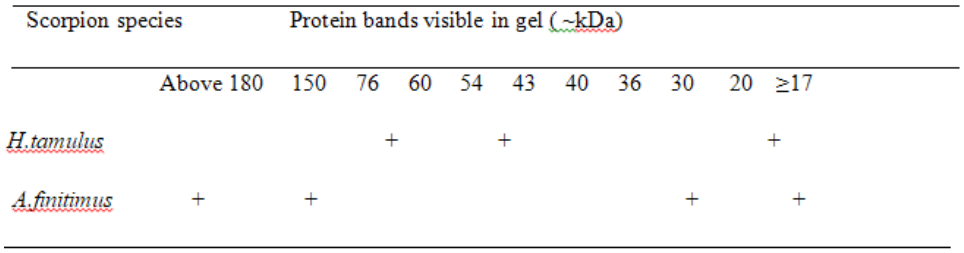

Table 2: Mass fingerprint of venom peptides of different fractions of *A. finitimus* by MALDI analysis.

Fractions		RT (min)	Molecular Identified masses m/z toxins					[M+H] +References
Fraction I	38.7	698.0	877.1					
Fraction II	47.9	764.3	1398.5	3177.2	4144.9	α-kTx 8.1	3177	Martin- Eauclaire and Bougis, 2012
Fraction III	55.1	1857.2						
Fraction IV	57.3	1857.2	4248.9					
Fraction V	80.6	3591.6	3747.5			TsKα[31]	3779.4	Pimenta et al., 2001
Fraction VI	83.9	3757.5	3775.6	7314.0		TsKα[31]	3779.4	Pimenta et al., 2001 Dhawan et al., 2002
						BtITx3	3796	
Fraction VII	94.2	5458.4	5795.4	6527.5		Birtoxin	6543.6	Inceoglu et al., 2001
Fraction VIII	96.3	666.1	877.1					
Fraction IX	112. 5	5845.2	6713.7	7458.9		TsIV47,48	7447.4	Pimenta et al., 2001

Note: RT: retention time. Molecular mass of main peak in each fraction is underlined and nearest to the previously identified toxins.

results of bodyweight during the experimental period for all studied groups. Bodyweight was significantly decreased (P<0.0001) in the positive control group compared to the negative control group during the investigative time. Meanwhile, the bodyweight was gradually increased till the end of the treatment period in venom-treated groups (300 and 150 mg/kg), and metformin treated group compared to the positive control group (P<0.0001). Effect of venom of *Hottentota tamulus* and *Androctonus finitimus* on body weight is described in Tables 2 and 3 respectively.

Table 4 describes the results of body weight when fractions of *Hottentota tamulus* venom HF1 1.3mg/kg (fraction III-3573 Da), HF2 1.4mg/kg (fraction V-3727.5 Da), HF3 1.5mg/kg (fraction VII3525 Da) were applied on the experimental groups. During this experiment, the body weight was gradually increased till the end of the treatment period in HF1 and HF2 (*Hottentota tamulus* fractions) treated groups but in HF3 treated groups body weight was not increased.

The Table 5 shows the body weight outcomes after applying *Androctonus finitimus* venom fractions AF1 1.3mg/kg (fraction V-3747.5 Da), AF2 1.4mg/kg (fraction VI-7315 Da), AF3 1.4mg/kg (fraction VII-5795.4 Da) to the experimental groups. AF1 and AF2 (*Androctonus finitimus* fractions) showed effective results in increasing body weight, while AF3 did not show any increase in body weight.

Effect of scorpion venom on fasting blood glucose level

Tables 5 and 6 shows the results of fasting blood glucose level during the experimental period of all studied groups. The fasting blood glucose levels were significantly increased in the positive control group than the negative control group during the experimental period. Meanwhile, the levels of fasting blood glucose were slowly decreased till the end of the treatment period in venom-treated groups (300 and 150 mg/kg) and metformin treated group compared to the positive control group (P<0.0001). Effect of venom of *Hottentota tamulus* and *Androctonus finitimus* on fasting blood glucose level is

Table 3: Effect of venom of *Hottentota tamulus* scorpion on body weight.

Groups	Negative control	Positive Control	Crude venom treated Group (300 mg/kg)	50% diluted venom treated Group (150 mg/kg)	Metformin Treated group (1.8 mg/kg)	P value
Initial	268.7 ± 5.8^{c}	134.5 ± 1.55	147.5 ± 1.88	157.4 ± 2.58^{c}	149.5 ± 2.31^{a}	<0.0001
% change	---------	-49.94%	9.66%	17%	11.15%	
1st week	281.9 ± 7.65^{c}	132.1 ± 1.38	151.5 ± 1.91^{a}	163.9 ± 4.2^{c}	156.2 ± 2.59^{b}	
% change	---------	-53.14%	14.68%	24.07%	18.24%	
2nd week	291.7 ± 8.76^{c}	127 ± 1.6	154.2 ± 2.17^{b}	166 ± 4.3^{c}	158.3 ± 2.49^{c}	
% change	--------	-56.46%	21.41%	30.7%	24.64%	
3rd week	303.4 ± 9.77^{c}	119.8 ± 1.31	160.1 ± 2.92^{c}	169 ± 4.54^{c}	160.7 ± 2.49^{c}	
% change	--------	-60.5%	33.63%	41.06%	34.14%	
4th week	314.9 ± 9.28^{c}	107.2 ± 2.07	165.5 ± 3.15^{c}	176 ± 4.9^{c}	170.3 ± 2.42^{c}	
% change	-------	-65.95%	54.3%	64.17%	58.86%	

Note: Initial means one day before administration of different treatments. Values are expressed in mean ± SEM, n=10. Statistical analysis was performed by one-way ANOVA test followed by Tukey's post hoc test. Values with different superscript letters represent significant differences (P<0.05). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 compared to positive control group.

Table 4: Effect of venom of Androctonus finitimus scorpion on body weight.

Groups	Negativecontrol	Positivecontrol	Crude venomtreated Group (300 mg/kg)	50% dilutedvenom treated Group (150 mg/kg)	MetforminTreated group (1.8 mg/kg)	P value
Initial	265.7 ± 5.7^{c}	137.5 ± 1.65	145.5 ± 1.78	159.4 ± 2.68^{c}	147.5 ± 2.51^{a}	<0.0001
% change	--------	-47.94%	11.66%	15%	13.15%	
1st week	278.9 ± 7.55^{c}	134.1 ± 1.48	150.5 ± 1.81^{a}	164.9 ± 4.3^{b}	154.2 ± 2.79^{a}	
% change	--------	-51.13%	16.69%	22.07%	20.24%	
2nd week	289.7 ± 8.66^{c}	129 ± 1.7	150.2 ± 2.47^{b}	170 ± 4.7^{c}	155.3 ± 2.59^{c}	
% change	--------	-53.56%	24.31%	32.7%	22.64%	
3rd week	300.4 ± 8.77^{c}	121.8 ± 2.31	158.1 ± 3.92^{c}	171 ± 4.44^{c}	159.7 ± 2.59^{c}	
% change	--------	-63.5%	30.53%	42.16%	33.14%	
4th week	317.9 ± 8.38^{c}	104.2 ± 3.17	167.5 ± 3.25^{c}	175 ± 4.9^{a}	171.3 ± 2.42^{b}	
% change	-------	-63.95%	50.3%	61.17%	56.76%	

Note: Initial means one day before administration of different treatments. Values are expressed in mean ± SEM, n=10. Statistical analysis was performed by one-way ANOVA test followed by Tukey's post hoc test. Values with different superscript letters represent significant differences (P<0.05). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 compared to positive control group.

Table 5: Effect of venom fractions (*Hottentota tamulus*) on body weight.

Groups	Negativecontrol	Positivecontrol	*Hottentotatamulus* Fraction 1(1.3 mg/kg)	*Hottentotatamulus* Fraction 2(1.4 mg/kg)	*Hottentotatamulus* Fraction 3(1.5 mg/kg)	P value
Initial	268.7 ± 5.8^{c}	134.5 ± 1.55	145.5 ± 1.78	160.4 ± 2.58^{c}	130.5 ± 2.31^{a}	<0.0001
% change	-------	-49.94%	10.66%	18%	11.15%	
1st week	281.9 ± 7.65^{c}	132.1 ± 1.38	161.5 ± 1.81^{a}	167.9 ± 4.2^{c}	120.2 ± 2.59^{b}	
% change	------	-53.14%	15.68%	25.07%	18.24%	
2nd week	291.7 ± 8.76^{c}	127 ± 1.6	165.2 ± 2.27^{b}	172 ± 4.3^{c}	115.3 ± 2.49^{c}	
% change	-------	-56.46%	23.41%	31.7%	20.64%	
3rd week	303.4 ± 9.77^{c}	119.8 ± 1.31	170.1 ± 2.92^{c}	177 ± 4.54^{c}	110.7 ± 2.49^{c}	
% change	-------	60.5%	35.63%	41.06%	30.14%	
4th week	314.9 ± 9.28^{c}	107.2 ± 2.07	175.5 ± 3.25^{c}	181 ± 4.9^{c}	112.3 ± 2.42^{c}	
% change	-------	-65.95%	56.3%	65.17%	45.86%	

described in the Tables 5 and 6 respectively.

Figure 4 Graph A and B represents the effect of venom fractions on blood glucose level. when venom fractions from Table 1 (fraction III-3573 Da (1.3mg/kg), fraction V-3727.5 Da (1.4mg/kg) and fraction VII-3525 Da (1.5mg/kg) of *Hottentota tamulus* scorpion and venom fractions from Table 2 (fraction V-3747.5 Da (1.3mg/kg), fraction VI-7315 Da (1.4mg/kg) and fraction VII-5795.4 Da(1.5mg/kg) of *Androctonus finitimus* scorpion were applied. All fractions of *Hottentota tamulus* (HF1, HF2, HF3) decreased blood glucose level whereas 2 fractions of *Androctonus finitimus* (AF1, AF2) decreased blood glucose level but 3rd fraction AF3 increased blood glucose level (Table 7).

Table 6: Effect of venom fractions (*Androctonus finitimus*) on body weight.

Groups	Negativecontrol	Positivecontrol	*Androctonusfinitimus* Fraction 1 (1.3 mg/kg)	*Androctonusfinitimus* Fraction 2 (1.4 mg/kg)	*Androctonusfinitimus* Fraction 3 (1.5 mg/kg)	P value
Initial	265.7 ± 5.7[c]	137.5 ± 1.65	150.5 ± 1.88	164.4 ± 2.68[c]	125.5 ± 2.51[a]	<0.0001
%change	--------	-47.94%	12.66%	16%	13.15%	
1st week	278.9 ± 7.55[c]	134.1 ± 1.48	161.5 ± 1.81[a]	170.9 ± 4.3[b]	119.2 ± 2.79[a]	
%change	--------	-51.13%	17.69%	23.07%	17.24%	
2nd week	289.7 ± 8.66[c]	129 ± 1.7	170.2 ± 2.57b	175 ± 4.7[c]	111.3 ± 2.59[c]	
%change	--------	53.56%	25.31%	33.7%	19.64%	
3rd week	300.4 ± 8.77[c]	121.8 ± 2.31	165.1 ± 3.92[c]	176 ± 4.44[c]	113.7 ± 2.59[c]	
%change	--------	-63.5%	31.53%	42.16%	29.14%	
4th week	317.9 ± 8.38[c]	104.2 ± 3.17	172.5 ± 3.25[c]	181 ± 4.9[a]	101.3 ± 2.42[b]	
%change	--------	-63.95%	51.3%	62.17%	50.76%	

Table 7: Effect of venom of scorpion *Hottentota tamulus* on fasting blood glucose level.

Groups	Negative Control	Positive Control	Crude Venom Treated group (300 mg/kg)	50% diluted Venom treated group (150 mg/kg)	Metformin Treated group (1.8 mg/kg)	P value
Initial	97.4 ± 2.04[a]	479.3 ± 16.6	460.9 ± 14.36	465.7 ± 6.49	455.8 ± 10.6	<0.0001
% change	------	392%	4.7%	1.13%	0.7%	
1st week	98.9 ± 2.12[b]	486.9 ± 21.6	450.8 ± 22.4[a]	368.3 ± 11[c]	355.5 ± 12.5[a]	
% change	------	392.3%	7.41%	24.35%	26.98%	
2nd week	95.6 ± 3.01[c]	499.5 ± 23.7	346.5 ± 14.2[b]	288.4 ± 7.5[c]	266.6 ± 17.6[b]	
% change	------	422.48%	32.65%	42.26%	46.6%	
3rd week	101 ± 3.3[c]	514.7 ± 16.9	236.7 ± 11.35[c]	181 ± 7.06[c]	174.2 ± 6.7[c]	
% change	-------	365.34%	54%	64.83%	66.15%	
4th week	100.4 ± 3.5[c]	520.6 ± 19.9	157.9 ± 5.12[c]	118.9 ± 2.7[c]	109 ± 3.23[c]	
% change	-------	418.5%	69.6%	77.16%	79.06%	

Note: Initial means one day before administration of different treatments. Values are expressed in mean ± SEM, n=10. Statistical analysis was performed by one-way ANOVA test followed by Tukey's post hoc test. Values with different superscript letters represent significant differences (P<0.05). [a]P<0.05, [b]P<0.01, [c]P<0.001compared to positive control group.

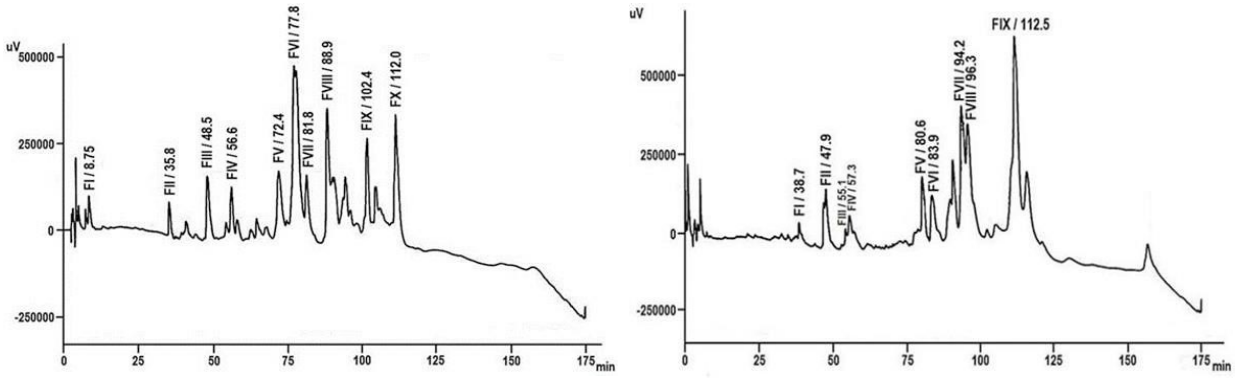

Figure 4: HPLC chromatographs of *H. tamulus* (A) and *A. finitimus* (B) venom. Approximately 6 mg crude venom was fractionated on C 18 column by reverse phase HPLC at the flow rate of 1 ml/min for 180 minutes. Fractions were collected and lyophilized. Major fractions are labeled and further analyzed by MALDI-TOF MS.

Effect of venom on level of insulin and lipid profile

Tables 8 and 9 display the results of plasma insulin and lipid pro-file values of all investigated groups. For diabetic rats, a substantial increase in triacylglycerol, total cholesterol, LDL-C and a significant reduction (P<0.0001) in HDL-C and plasma insulin levels were observed compared to the negative control. Treatment with venom using two doses (300 and 150 mg/kg) and of metformin significantly increased (P<0.0001) the undesirable changes in insulin and lipid profile compared to the positive control group (Table 10).

Figure 5 Graph A and Graph B represent effect of the venom fractions of scorpion *Hottentota tamulus* (fraction III-3573 Da (1.3mg/kg), fraction V-3727.5 Da (1.4mg/kg), fraction VII-3525 Da(1.5mg/kg) and *Androctonus finitimus* (fraction V-3747.5 Da(1.3mg/kg), fraction VI-7315 Da (1.4mg/kg), fraction VII-5795.4 Da(1.5mg/kg) on the level of mice blood insulin. Plasma insulin level increased when fraction III and fraction VII of *Hottentota tamulus* was applied and it was decreased during fraction V. Graph B represents that plasma insulin level increased in fraction V and fraction VI and it is decreased in fraction VII.

Figure 6 Graph (A) and (B) represent effect of venom fractions on cholesterol level. Cholesterol level decreased when fraction 1 and fraction 2 were applied rather cholesterol level increased when fraction 3 of both *Hottentota tamulus* and *Androctonus finitimus* venom were applied.

Histopathalogical effect of scorpion venom

Table 8: Effect of venom of scorpion *Androctonus finitimus* on fasting blood glucose level.

Groups	NegativeControl	PositiveControl	Crude Venomtreated group(300 mg/kg)	50% diluted Venom treated group (150 mg/kg)	MetforminTreated group (1.8 mg/kg)	P value
Initial	95.4 ± 2.14^{c}	481.3 ± 16.5	469.9 ± 14.36	467.7 ± 6.49	490.8 ± 8.6	<0.0001
% change	--------	382%	5.7%	2.13%	5.7%	
1st week	100.9 ± 2.12^{c}	484.9 ± 20.6	455.8 ± 22.4^{a}	365.3 ± 11.9^{c}	495.5 ± 12.5^{a}	
% change	-------	395.3%	7.71%	20.35%	34.98%	
2nd week	97.6 ± 3.21^{c}	497.5 ± 21.7	340.5 ± 12.2^{b}	291.4 ± 9^{c}	500.6 ± 19.6^{b}	
% change	-------	424.58%	30.55%	40.26%	55.6%	
3rd week	106 ± 3.4^{c}	510.7 ± 17.7	233.7 ± 9.35^{c}	184 ± 9.06^{c}	520.2 ± 6.7^{c}	
% change	-------	345.34%	65%	59.83%	65.15%	
4th week	95.4 ± 3.5^{c}	530.6 ± 19.9	149.9 ± 5.12^{c}	108.9 ± 2.78^{c}	545 ± 3.23^{c}	
% change	-------	421.5%	72.6%	77.16%	84.06%	

Note: Initial means one day before administration of different treatments. Values are expressed in mean ± SEM, n=10. Statistical analysis was performed by two-way ANOVA test followed by Tukey's post hoc test. Values with different superscript letters represent significant differences (P<0.05). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001compared to positive control group.

Table 9: Effect of venom of scorpion *Hottentota tumulus* on level of insulin and lipid profile.

Groups	NegativeControl	PositiveControl	Crude Venom treated group (300 mg/kg)	50%dilutedVenom treated group (150 mg/kg)	Metformintreated group (1.8 mg/kg)	P value
Insulin (pg/mL)	126 ± 1.16^{c}	92.9 ± 1.32	82.7 ± 2.03^{c}	100.6 ± 0.96^{c}	85.1 ± 1.14^{c}	<0.0001
% change	------	-54.05%	42.83%	35.01%	112.61%	
Triacylglycerol(mg/dL)	82.8 ± 1.75^{c}	227.7 ± 4.3	158.5 ± 2.3^{c}	235.7 ± 1.7^{c}	108 ± 1.43^{c}	
% change	--------	175%	-30.25%	-47%	-52.56%	
Total cholesterol(mg/dL)	84.3 ± 1.57^{c}	235.6 ± 2.8	166.9 ± 2.25^{c}	250.6 ± 1.52^{c}	96.5 ± 1.15^{c}	
% change	--------	179.47%	-29.15%	-55.6%	-59%	
LDL-C (mg/dL)	40.1 ± 1.32^{c}	178.24 ± 2.5	113.6 ± 2.32^{c}	184.9 ± 1.63^{c}	46.1 ± 1.13^{c}	
% change	--------	344.04%	-36.26%	-69.16%	-74.13%	
HDL-C (mg/dL)	27.6 ± 0.47^{c}	35.7 ± 0.34	21.6 ± 0.45^{c}	55.5 ± 0.75^{c}	28.8 ± 0.62^{c}	
% change	--------	-57.6%	84.6%	117.9%	146.15%	

Note: Values are expressed in mean ± SEM, n=10. Statistical analysis was performed by one-way ANOVA test followed by Tukey's post hoc test. Values with different superscript letters represent significant differences (P<0.05). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 compared to positive control group.

Table 10: Effect of venom of scorpion *Androctonus finitimus* on level of insulin and lipid profile.

Groups	NegativeControl	PositiveControl	Crude Venom treated group (300 mg/kg)	50%dilutedVenom treated group (150 mg/kg)	Metformintreated group (1.8 mg/kg)	P value
Insulin (pg/mL)	124 ± 1.14^{a}	59.9 ± 1.34	79.7 ± 2.03^{c}	123.6 ± 0.96^{b}	47.1 ± 1.14^{a}	<0.0001
% change	--------	-50.35%	44.53%	115.01%	95.61%	
Triacylglycerol(mg/dL)	85.8 ± 1.55^{b}	225.7 ± 6.3	230.5 ± 2.5^{c}	124.7 ± 1.5^{b}	110 ± 1.33^{a}	
% change	-------	179%	-35.25%	-45%	-50.56%	
Total cholesterol(mg/dL)	80.3 ± 1.67^{c}	238.6 ± 2.7	250.9 ± 2.35^{c}	109.6 ± 1.42^{b}	93.5 ± 1.25^{b}	
% change	--------	175.47%	-25.15%	-55.6%	-57%	
LDL-C (mg/dL)	43.14 ± 1.2^{c}	175.24 ± 3.5	190.6 ± 2.32^{c}	57.96 ± 1.53^{c}	45.1 ± 1.23^{b}	
% change	--------	345.04%	-35.26%	-65.16%	-72.13%	
HDL-C (mg/dL)	25.6 ± 0.47^{c}	14.7 ± 0.34	25.6 ± 0.45^{c}	10.5 ± 0.75^{c}	12.8 ± 0.62^{c}	
% change	-------	-56.6%	85.6%	121.9%	140.15%	

Note: Values are expressed in mean ± SEM, n=10. Statistical analysis was performed by two-way ANOVA test followed by Tukey's post hoc test. Values with different superscript letters represent significant differences (P<0.05). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 compared to positive control group.

The microscopic histological examinations (H&E) of pancreatic tissue of control and treated mice groups are illustrated in Figure 7. Histological changes in islets of Langerhans of mice pancreas after treatment intraperitoneally with venom of scorpion *Hottentota tamulus* and *Andrctonus finitimus* for five 5 weeks showing the difference of islets size. A. Section from control group showing granulated cytoplasm of islet cells ; B. Section after treatment treated with venom of 150mg/kg cells began to recover ;C Section from venom treated group (300mg/kg) presenting recovery of beta cells of pancreas; D Section from metformin treated group showing appearent recovery of cells presenting regular outline of an islet. Figure 7A representing *Hottentota tamulus* venom and Figure 7B representing *Androctonus finitimus* venom.

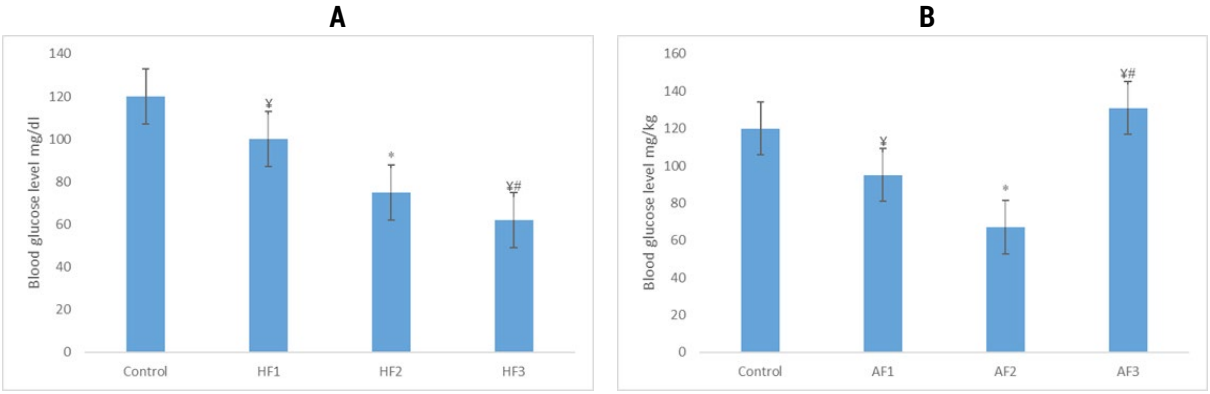

Figure 5: Graph A and B represent the effect of venom fractions on blood glucose level.

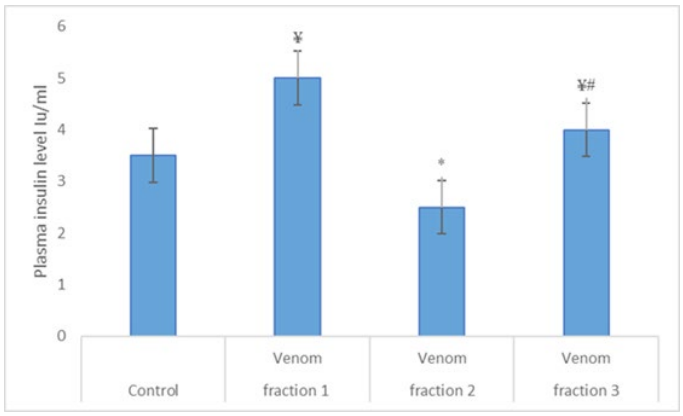

Figure 5A: Plasma insulin level of *Hottentota tamulus*.

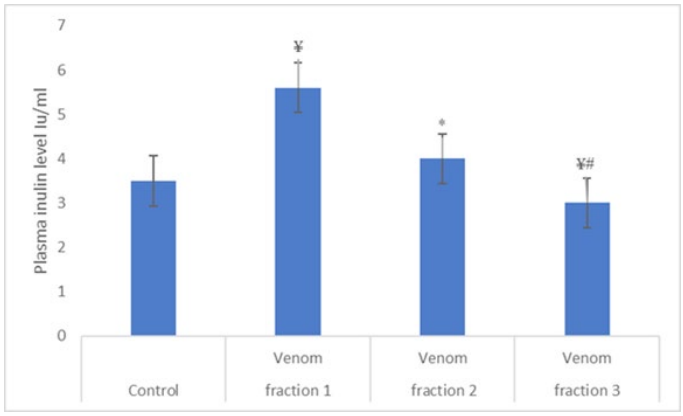

Figure 5B: Plasma insulin level of *Androctonus finitimus*.

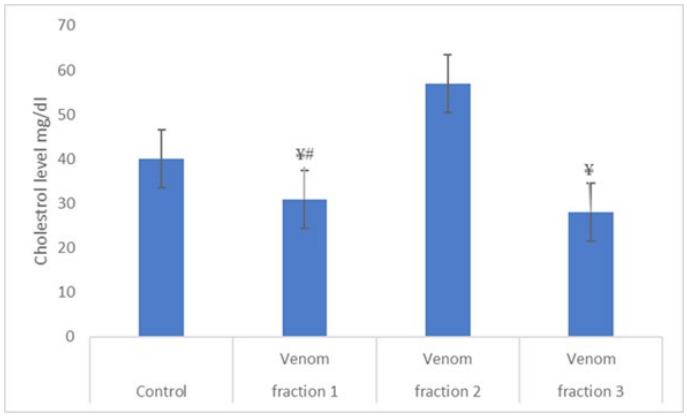

Figure 6: (A) Effect of venom fractions of *Hottentota tamulus* on cholesterol level.

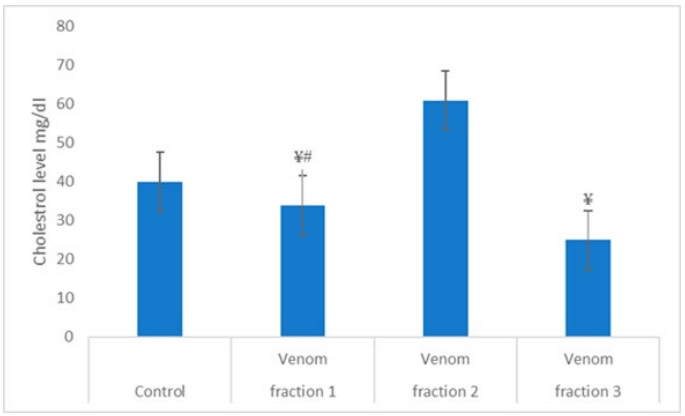

Figure 6: (B) Effect of venom fractions of *Androctonus finitimus* on cholesterol level.

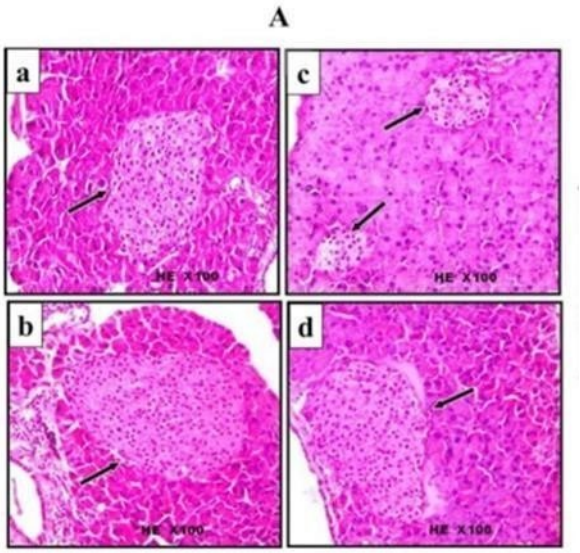

Figure 7A: Section A is from control group; section b is from venom (*Hottentota tamulus)* treated group (150 mg/kg); section c is from venom (*Hottentota tamulus*) treatedgroup (300 mg/kg); section d is from metformin treated group.

Discussion

Diabetes mellitus is an endocrine disorder followed by diverse metabolic disturbances [9] and characterized by hyperglycemia and disruption of carbohydrate, fat, and protein metabolism associated with an absolute or relative deficiency in insulin secretion or insulin action. Hyperglycemia, hyperlipidemia, hypertension, atherosclerosis, retinopathy, neuropathy, and nephropathy are the basic issues of DM [10].

Antidiabetic medications include a number of negative side effects when used for an extended period of time, including stomach pain, hypoglycemia,

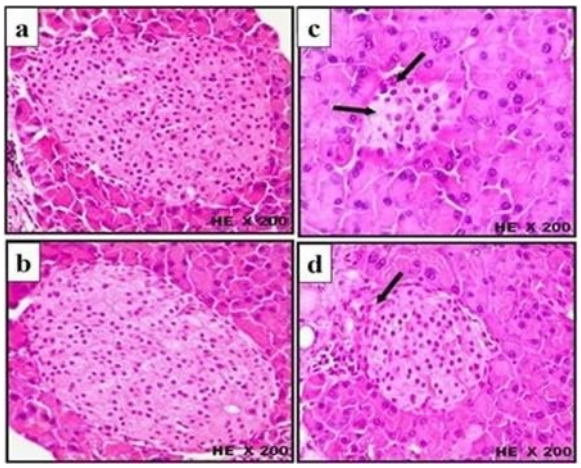

Figure 7B: Section A is from control group; section b is from venom (*Androctonus finitimus*) treated group (150 mg/kg); section c is from venom (*Androctonus finitimus)* treated group (300 mg/kg); section d is from metformin treated group.

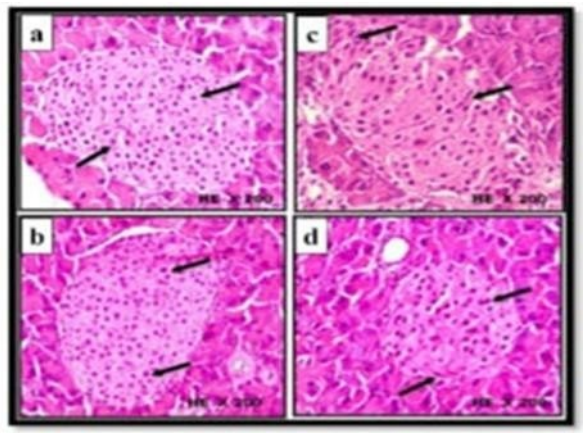

Figure 7C: Histological changes in pancreas after treatment intraperitoneally with scorpion venom fractions for five weeks (i) Negative control group (ii) HFI (iii) HFII (iii) HFIII.

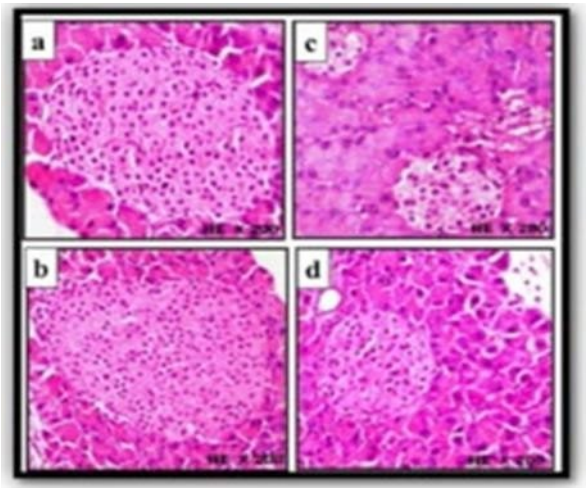

Figure 7D: Histological changes in pancreas after treatment intraperitoneally with scorpion venom fractions for five weeks (i) Negative control group (ii) AFI (iii) AFII (iii) AFII.

nausea, headache, and weight. As a result, innovative anti-diabetic medicines with great therapeutic efficacy and low toxicity are required. Using an alloxan-induced diabetic mice model, the current study investigates the possible antidiabetic effectiveness of *Hottentota tamulus* and *Androctonus finitimus* venom. Alloxan (a routinely used hyperglycemic drug in experimental animals and in the production of Type-1 DM) primarily penetrates beta-cells via the GLUT2 glucose transporter.

Alloxan has a severe necrotizing impact on beta-cells due to its particular inhibition of glucokinase and formation of ROS. As a result, a reduction in the number of beta-cells resulted in insulin shortage, which was followed by numerous metabolic problems in carbohydrates, proteins, and lipids [11]. In diabetic rats, the deteriorating effect of alloxan on pancreatic β-cells caused reduction in insulin secretion, hyperglycemia, and hyperlipidemia consequently [12]. In the recent experiment, the glucose level was substantialy increased in the diabetic group and resulted in significant decrease in plasma insulin level in comparison to the normal control group. These results may be due to the cytotoxic effect of alloxan which causes the damages of islets of Langerhans and significantly reduced insulin secretion in diabetic rats compared to the normal control group [13]. Similar studies had confirmed the hypoglycemic activity of venom in diabetic rats, respectively, which is consistent with our findings [14,15].

The findings clearly showed that injecting scorpion venom fractions considerably raised the level of plasma insulin. The hypoglycemic properties of scorpion venom might be ascribed to (i) stimulation of pathways involved in β-cell renewal [16]; (ii) enhancing the activity of enzymes responsible for glucose utilization by insulin-dependent pathways and (iii) secretion of insulin from β-cells [17]. Histopathological investigations corroborated these findings, which was surprising. Scorpion venom enhanced the morphological and cellular properties of pancreatic tissue while increasing the quantity and size of cells [18] and decrease volum of pancreatic islets β-cells. Similar results were obtained by Xie and colleagues [19]. They reported hypoglycemic activity of scorpion extract (Chinese scorpion of B. martensii kirsch) mixed with gypsum in streptozotocin-induced diabetic mice. The activation of PPARγ restores the function of beta-cell in diabetic mice through (i) reducing stress on endoplasmic reticulum; (ii) protecting structure of euchromatin and (iii) regulation the transcription of PDX-1 [20,21]. PDX-1 regulates the repairment of pancreas and differentiation of beta cells [18].

The results showed that the positive control group had a substantial rise in fasting blood glucose levels and a significant drop in plasma insulin levels when compared to the negative control group. Meanwhile, venom-treated groups employing two dosages (300 and 150 mg/kg) plus metformin demonstrated a significant decrease in fasting blood glucose levels and a significant increase in plasma insulin levels when compared to the positive control group. These results are in line with other studies [14,22,23] that confirmed the hypoglycemic activity of bee venom and its ability to raise insulin secretion.

The results showed a substantial increase in total cholesterol, triacylglyceride, LDL-C, and a significant decrease in HDL-C levels in the positive control group compared to the negative control group at the conclusion of the study period. These findings are congruent with those of previous investigations [24], which reported that the levels of triacylglycerol, total cholesterol, LDL-C, and HDL-C were significantly decreased in diabetic hyperlipidemic rats, whereas the groups treated with scorpion venom at both doses (300 and 150 mg/kg) and metformin showed a significant decline in total cholesterol, triacylglycerol, LDL-C, and a significant elevation in HDL-C levels [25]. The results are supported by other studies [26,27] which suggested the ability of venom to reduce lipid profile abnormalities through increasing insulin secretion and sensitivity in experimental animal models.

Histopathological data obtained from the current study were also in consistent with [28] who found that the size and quantity of pancreatic islets were decrease in diabetic rats compared to normal rats, but venom-treated animals demonstrated regeneration of both islets. The restoration of the histological structure of the pancreas may be linked to venom components, which suppressed pancreatic β-cell inflammation and hence enhanced insulin output [29,30]. These results in harmony who confirmed hypoglycemic activity of scorpion venom in diabetic rats.

Conclusion

The findings of this study displayed that body weight of mice increased when alloxan was injected and after applying venom of scorpions and dose of metformin there was significant decrease in body weight of mice. The results showed a substantial increase in total cholesterol, triacylglyceride , LDL-C,

and a significant decrease in HDL-C levels in treated groups as compared to control groups. The results showed that the treated groups had a substantial rise in fasting blood glucose levels when compared to control group. Scorpion venom protects against biochemical and histological alterations in the islets of Langerhans in diabetic rats.

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