Original Research

Bee venom ameliorates cardiac dysfunction in diabetic hyperlipidemic rats

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Impact statement

Diabetes and hyperlipidemia are associated with a significantly higher risk of cardiovascular diseases. The present study describes the cardiovascular complications in diabetic hyperlipidemic rats with an emphasis on the importance of using natural products such as bee venom to treat cardiovascular diseases. Bee venom can attenuate cardiac dysfunction in diabetic hyperlipidemic rats in a dose-dependent manner compared to the combined therapy of metformin and atorvastatin. Instead of synthetic drugs, bee venom can be used as an alternative therapy for treating diabetes associated with hyperlipidemia.

Abstract

High levels of blood glucose and lipids are well-known risk factors for heart diseases. Bee venom is a natural product that has a potent hypoglycemic, hypolipidemic, anti-inflammatory, and antioxidant effects. The current study aimed to determine the bee venom effects on cardiac dysfunction compared to combined therapy of metformin and atorvastatin in diabetic hyperlipidemic rats. The median lethal dose of bee venom was estimated, and then 50 adult male albino rats were categorized into five groups. One group was fed a standard diet and served as a negative control, while the other groups were given nicotinamide and streptozotocin injections to induce type 2 diabetes. After confirming diabetes, the rats were fed a high-fat diet for four weeks. The four groups were divided as follows: one group served as a positive control, whereas the other three groups were treated with bee venom (0.5 mg/kg), bee venom (1.23 mg/kg), and combined therapy of metformin (60 mg/kg) and atorvastatin (10 mg/kg), respectively, for four weeks. Upon termination of the experiment, blood samples and heart tissue were obtained. Administration of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy of metformin and atorvastatin revealed a significant decrease in the concentrations of glucose, total cholesterol, triacylglycerol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, troponin I, creatine kinase, and lactate dehydrogenase activities. Moreover, a significant decrease had been detedcted in malondialdehyde, nuclear factor-kappa-β levels, and relative mRNA expression of vascular cell adhesion molecule-1 and galectin-3 in heart tissue compared to the positive control ($P < 0.0001$). Furthermore, there was a significant increase in bodyweight levels of insulin, high-density lipoprotein cholesterol, and total antioxidant capacity in heart tissue compared to the positive control ($P < 0.0001$). The results indicate that bee venom can ameliorate cardiac dysfunction through attenuating oxidative stress and downregulating the NF-$\kappa\beta$ signaling pathway.

Keywords: Heart, bee venom, diabetes, hyperlipidemia, oxidative stress, NF-$\kappa\beta$, VCAM-1, galectin-3

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Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder defined by persistent hyperglycemia, deficiency in insulin secretion and insulin response defects.[1]

Diabetes mellitus contributes to quality-of-life impairments, including cardiovascular diseases (CVDs), retinopathy, which can lead to blindness, a renal failure because of diabetic nephropathy (DN), autonomic neuropathy, that can result in bowel and bladder dysfunction risk, and foot ulcers risk and difficulty walking.[2–5]

Hyperlipidemia is a metabolic disease described by elevated levels of triacylglycerol (TAG), total cholesterol (TC), and low-density lipoprotein-cholesterol (LDL-C), and reduced levels of high-density lipoprotein-cholesterol (HDL-C).[6] The presence of hyperlipidemia, along with diabetes mellitus, increases the chance of developing cardiovascular disease.[7]

The association between diabetes mellitus, hyperlipidemia, and cardiovascular diseases can be attributed to oxidative stress and inflammation.[8] Excess reactive oxygen species induce nuclear factor kappa (NF-$\kappa\beta$) activation, promoting the transcription of inflammatory mediators and triggers myocardial inflammation.[9]

In the development of alternative therapies for many illnesses, much attention has been given to natural products, including certain species' venom.[10] Bee venom consists of low molecular ingredients, enzymes, and peptides. These peptides include adolapin, apamin, melittin, and mast cell degranulating peptide. Hyaluronidase, phosphatase, α-glucosidase, phospholipaseA2 (PLA2), and phospholipase B enzymes are included. In addition to some carbohydrates, non-peptides like histamine, dopamine, and norepinephrine are also present in bee venom components.[11]

The use of bee venom in the treatment of a variety of diseases has shown to be effective.[12] Several studies have shown bee venom is utilized as a hypoglycemic, hypolipidemic, anti-inflammatory, and antioxidant agent.[13–16]

Melittin, apamin, and phospholipase A2 are the key components of bee venom. Individual components, as well as the whole bee venom, have been shown to exhibit myocardium protective effects through regulation of inflammation, magnesium ions, calcium ions, and activation of peroxisome proliferator-activated receptor-α and -γ. Phospholipase A2 inhibits lipid accumulation in the aortic valve and aorta, and protects against atherosclerosis by suppressing the transition of macrophages into foam cells.[17]

This research aims to determine the bee venom effects on cardiac dysfunction compared to the combined therapy of metformin and atorvastatin in diabetic hyperlipidemic rats.

Materials and methods

Bee venom

The Carniolan bee venom (*Apis mellifera carnica*) specimen was obtained from the Beekeeping Research Department of the Plant Protection Research Institute of the Agriculture Research Centre in Dokki, Giza governorate, Egypt.

Chemicals and drugs

Streptozotocin and nicotinamide were purchased from sigma chemicals Co. (St. Louis, Mo. USA). Metformin hydrochloride and atorvastatin calcium were obtained as a gift from Amoun pharmaceutical company, Egypt. Cholesterol, cholic acid, and tert. Butylhydroquinone were obtained from Alpha chemical company, India. Vitamins mixture, minerals mixture, and L-cystine were obtained from Acros-Organics Chemical Co. (USA).

Animals

Adult male albino rats weighing 150–170 g were housed in the animal house of the Faculty of Science, Zagazig University. Rats were kept under laboratory conditions of temperature (20–25°C), humidity (60–65%), and 12-h light/dark cycle. Rats had free access to tap water and a regular chow diet ad libitum. The Ethical Committee of Zagazig University approved the experimental design and animal handling (Approval number ZU-IAUUC/1/F/32/2019).

Determination of the median lethal dose of carniolan bee venom

The median lethal dose (LD_{50}) of carniolan bee venom was calculated by arranging ascending concentrations of five bee venom doses in regular progression, starting with a dose that kills approximately 0–20% of the animals and ending with a dose that kills approximately 80–100% of the injected animals. Each dose level was tested in five rats, and all injections were given intraperitoneally. Mortality and survival rates of injected animals were recorded after 24 h from the time of injection. The following equations were used to measure LD_{50} using a previous method:[18]

Log LD_{50} = log dose next below 50% + [log increasing factor × proportional distance]. Proportional distance = [50% – mortality next below]/[mortality next above – mortality next below].

Preparation of experimental diets

The American Institute of Nutrition designed the standard diet,[19] and the high-fat diet (HFD) had the same components as the standard diet with the addition of 23% fats, 1% cholesterol, and 0.2% cholic acid,[20] as shown in Table 1.

Type 2 diabetes induction in rats

Type 2 diabetes was induced in 18-h fasted rats (without food but allowed free access to water) with a single intraperitoneal streptozotocin (60 mg/kg) injection dissolved in 0.1 M citrate buffer (pH 4.5) after 15 min of intraperitoneal injection of nicotinamide (120 mg/kg) dissolved in saline.[21] After 1 h of streptozotocin and nicotinamide administration, rats were given 5% glucose solution overnight to prevent hypoglycemia. Fasting blood glucose was determined using a portable glucometer after 72 h and on the seventh day of injection. Rats had a fasting blood glucose above 250 mg/dL were considered diabetic.[22]

Table 1. Standard diet and high-fat diet composition.

Ingredients (g/100g diet)	Standard diet	High-fat diet
Corn starch	57.0692	32.8692
Casein	14	14
Sucrose	10	10
Soyabean oil	4	4
Cellulose	5	5
Mixture of vitaminsa	3.5	3.5
Mixture of mineralsa	1	1.0
L-cystine	0.18	0.18
choline bitartrate	0.25	0.25
Tert. Butylhydroquinone	0.0008	0.0008
Fats (tallow)	5	28
Cholesterol	0	1
Cholic acid	0	0.2

[a]Mixtures of minerals and vitamins that contributed to the diets were in line with the guidelines of American Institute of Nutrition.[19].

Hyperlipidemia induction

Diabetic rats were given a high-fat diet for four weeks to induce hyperlipidemia, while the negative control animals were fed a standard diet. The composition of experimental diets is presented in Table 1. At the end of the fourth week, about 1 mL of blood has been obtained from one of the lateral tail vein of rat using a 23-G needle.[23] Blood was collected in a free anti-coagulant tube to obtain serum in order to measure lipid profile to verify hyperlipidemia induction.[24] Subsequently, the high-fat diet was given along with treatment administration for four weeks.

Experimental design

After one week of acclimatization, 50 adult male albino rats were categorized into five groups (10/group) as listed below:

Group I: Negative control: Normal rats received saline solution by intraperitoneal injections daily for four weeks.
Group II: positive control: Diabetic hyperlipidemic rats.
Group III: Bee venom-treated group (0.5 mg/kg): Diabetic hyperlipidemic rats received bee venom (0.5 mg/kg) dissolved in distilled water by intraperitoneal injections daily for four weeks.[25]
Group IV: Bee venom-treated group (1.23 mg/kg): Diabetic hyperlipidemic rats received bee venom (1.23 mg/kg) dissolved in distilled water by intraperitoneal injections daily for four weeks. This dose was determined according to its LD_{50}, and 1/10th of LD_{50} (1.23 mg/kg) was taken as a therapeutic dosage.[26]
Group V: Metformin and atorvastatin-treated group: Diabetic hyperlipidemic rats received metformin (60 mg/kg) and atorvastatin (10 mg/kg) dissolved in distilled water orally administrated daily for four weeks.[27]

Monitoring of bodyweight and blood glucose throughout the treatment period

The bodyweights and fasting blood glucose rates were recorded weekly throughout the treatment period. The doses of bee venom, metformin, and atorvastatin were adjusted weekly to maintain the same dosage during the treatment period.

Specimen collection

Upon termination of the experiment, rats were fasted for 10 h, and all rats were euthanized by urethane. A drop of blood obtained from each rat tail-tip was utilized for measuring fasting blood glucose. Ten blood specimens from each group were obtained from the retro-orbital venous plexus in two tubes: one containing EDTA to get plasma via centrifugation at 4000 r/min for 20 min to estimate the insulin concentration, and the other is a free anti-coagulant tube to get serum via centrifugation at 4000 r/min for 20 min to evaluate the lipid profile and heart function tests. All these specimens were kept at −20°C until biochemical analysis.[28] Heart tissue was excised from each rat, rinsed in saline solution, cleared off blood, and cut into three parts. The first part (0.2 g) from each rat's heart tissue was homogenized in 2 mL phosphate buffer saline (pH 7.4) using a Teflon homogenizer, and then the homogenate was centrifuged at 3500 r/min for 15 min at 4°C.[29] The supernatants were used to determine the concentration of total antioxidant capacity, malondialdehyde, and nuclear factor-kappa-β for histopathological examination. The second portion of the heart tissue was immersed in 10% formalin solution, whereas the third part of heart tissue was kept at −80°C until RNA isolation.[30]

Biochemical parameters

Fasting blood glucose

Fasting blood glucose (FBG) was measured using a portable glucometer (Right Tango, TD-4235, Taiwan) according to the method in literature.[31]

Plasma insulin

Plasma insulin was estimated according to a previously described method[32] using an ELISA kit derived from Cloud-Clone Crop, USA.

Lipid profile

Serum triacylglycerol (TAG), total cholesterol (TC), and HDL-C were measured according to previous methods,[33–35] using commercial kits purchased from Spectrum Diagnostics Company, Egypt. LDL-C was calculated by Friedewald's equation: LDL-C = TC- (TAG/5+ HDL-C).[36] VLDL-C was calculated by Nobert's equation: VLDL-C = TAG/5.[37]

Serum creatine kinase, lactate dehydrogenase, and troponin I

Serum creatine kinase (CK-MB) was estimated according to a previous method[38] using commercial kits derived from Spectrum Diagnostics company, Egypt. Serum lactate dehydrogenase (LDH) was evaluated according to the method[39] using commercial kits purchased from Spinreact, Spain. Serum troponin I (TnI) was estimated according to a previous method[40] using ELISA kits derived from Eagle Biosciences company, USA.

Total antioxidant capacity, malondialdehyde, and nuclear factor-kappa-β

Total antioxidant capacity (TAC) and malondialdehyde (MDA) were estimated in heart tissue homogenate according to previous methods,[41,42] using a commercial kit purchased from Biodiagnostic Company, Egypt. Nuclear factor-kappa-β (NF-$\kappa\beta$) was assessed in heart tissue homogenate based on an earlier method[43] using an ELISA kit derived from Cloud-Clone Crop, USA.

Quantification real-time polymerase chain reaction

RNA was extracted from six heart tissue samples in each group using TRIzol™ Reagent (Invitrogen, USA) according to the manufacturer's protocol. The concentration and purity of the extracted RNA were estimated by measuring the absorbance at 260/280 nm using Beckman dual spectrophotometer, USA. RNA specimens with A260/A280 ratio between 1.9 and 2.1 were utilized for further analysis. A high-capacity cDNA synthesis kit (Qiagen, USA) was used to reverse transcribe 2 μg of extracted RNA into cDNA. Relative mRNA expression of vascular cell adhesion molecule-1(VCAM-1) and Galectin-3 was estimated by quantitative real-time PCR using SYBR Green Master Mix kit (Qiagen, Germany) using a real-time PCR System (Biometra, Germany). The real-time PCR thermal cycler was programmed as follows: 95°C for 5 min, 40 cycles (95°C for 10 sec, 55°C for 10 s, and 72°C for 30 s), and finally 72°C for 5 min. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was utilized as a housekeeping gene. The primer sequence of studied genes is listed in Table 2. The $2^{-\Delta\Delta ct}$ formula determined the relative expression of the amplified products in the target genes.[44]

Histopathological examination

Six samples of heart tissue from each group were used for histopathological examination. A piece of heart tissue was immersed for 24 h in 10% formalin for tissue fixation. The fixed tissues have been sectionalized, deparaffinized, rinsed, and stained with H&E for histopathological examination.[45]

Quantification analysis of area percentage (area %) of abnormal myocytes

Area percentage (Area %) of abnormal myocytes (Cell Number/Specific area) was evaluated in six non-overlapping fields from different heart sections of each group. Cell numbers were calculated as the number of cells per cross-sectional area using some features (cell counter/color deconvolution/color threshold) of the Image J program (version 1.6.0_20, National Institutes of Health, Bethesda, MD).[46] They were used for the evaluation of area percentage (Area %) of abnormal myocytes in different heart sections.

Statistical analysis

The statistical analysis was done by the Social Science Package version 25 (SPSS).[47] Normality of the data was first tested using Shapiro–Wilk test. The data were shown as mean ± SEM. The statistical significance was determined using two-way ANOVA test followed by Tukey's post hoc test. A value of $P < 0.05$ is statistically significant.

Results

The median lethal dose (LD_{50}) of carniolan bee venom

The median lethal dose (LD_{50}) of carniolan bee venom was 12.3 mg/kg by calculating the results in Table 3.

Serum lipid profile levels after hyperlipidemia induction

Table 4 summarizes the results of the lipid profile after consumption of standard and high-fat diets for four weeks.

Table 2. Primer sequences and gene bank accession number of studied genes.

Genes	Primer sequences	Gene bank accession number
VCAM-1	Forward 5′-TAAGTTACACAGCAGTCAAATGGA-3′ Reverse 5′-CACATACATAAATGCCGGAATCTT-3′	NM_012889.1
Galectin-3	Forward 5′-ATGGCAGACGGCTTCTCACT-3′ Reverse 5′-CGCGAAGGGTGCGGTACTAG-3′	KF639958.1
GAPDH	Forward 5′-ACCACAGTCCATGCCATCAC-3′ Reverse 5′-TCCACCACCCTGTTGCTGTA -3′	XM_032909104.1

Table 3. Carniolan bee venom toxicity in rats.

Dose mg/kg (a)	Dead (b)	Alive (c)	Total (d) Dead (d_1)	Total (d) Alive (d_2)	Percent of mortality (e) (%)
10.9	1	4	1	10	9.09
11.9	2	3	3	6	33.33
12.9	3	2	6	3	66.66
14.1	4	1	10	1	90.9
15.4	5	0	15	0	100

Note: Column (a) represents tested doses. The numbers of dead and alive rats after 24 h of doses injection are entered in column (b) and (c). Column (d) represents the accumulated number of dead and alive rats at given doses. Column (b) is therefore added from the top, and the subtotal for each dose is entered in column (d_1) as the accumulated number of dead rats at given doses plus those lower doses. Column (c) is therefore added from the bottom, and the subtotal for each dose is entered in column (d_2) as the accumulated number of alive rats at given doses plus higher doses. Percent of mortality is calculated from column (d_1 and d_2) and is entered in column (e).

Triacylglycerol, total cholesterol, LDL-C, and VLDL-C showed a significant elevation, while HDL-C levels showed a significant reduction in the positive control group compared to the negative control group ($P < 0.0001$).

Table 4. Serum lipid profile levels after hyperlipidemia induction.

Groups	Negative control	Positive control	*P* value
Triacylglycerol (mg/dL)	70.5 ± 1.29^c	181.9 ± 1.8	<0.0001
% change	———	158%	
Total cholesterol (mg/dL)	71.7 ± 1.36^c	189.3 ± 1.58	
% change	———	164%	
LDL-C (mg/dL)	29.3 ± 1.07^c	135.12 ± 2.3	
% change	———	361.2%	
VLDL-C (mg/dL)	14.1 ± 0.25^c	36.38 ± 0.36	
% change	———	158%	
HDL-C (mg/dL)	28.3 ± 0.66^c	19.1 ± 0.43	
% change	———	−32.5%	

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 ($P < 0.05$). $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control.

Effect of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on bodyweight and blood glucose

Table 5 summarizes the results of bodyweight monitoring during the experimental period for all studied groups. Bodyweight was significantly reduced ($P < 0.0001$) in the positive control group compared to the negative control group during the experimental period. Meanwhile, the bodyweight was gradually increased till the end of the treatment period in bee venom-treated groups (0.5 and 1.23 mg/kg), and metformin and atorvastatin-treated group compared to the positive control group ($P < 0.0001$).

Table 6 presents the results of fasting blood glucose monitoring during the experimental period of all studied groups. The fasting blood glucose levels were significantly elevated in the positive control group than the negative control group during the experimental period. Meanwhile, the levels of fasting blood glucose were gradually diminished till the end of the treatment period in bee venom-treated groups (0.5 and 1.23 mg/kg) and metformin and atorvastatin-treated group compared to the positive control group ($P < 0.0001$).

Table 5. Effect of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on body weight.

Groups	Negative control	Positive control	Bee venom treated group (0.5 mg/kg)	Bee venom treated group (1.23 mg/kg)	Metformin- and atorvastatin-treated group	*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 ± 1.67	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%	

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 ($P < 0.05$). $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

Table 6. Effect of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on fasting blood glucose levels.

Groups	Negative control	Positive Control	Bee venom-treated group (0.5 mg/kg)	Bee venom-treated group (1.23 mg/kg)	Metformin- and atorvastatin-treated group	*P* value
Initial	97.4 ± 2.04^c	479.3 ± 16.6	501.9 ± 14.36	484.7 ± 6.49	482.8 ± 10.6	< 0.0001
% change	———	392%	4.7%	1.13%	0.7%	
1st week	98.9 ± 2.12^c	486.9 ± 21.6	450.8 ± 22.4^a	368.3 ± 11.9^c	355.5 ± 12.5^c	
% change	———	392.3%	−7.41%	−24.35%	−26.98%	
2nd week	95.6 ± 3.01^c	499.5 ± 23.7	336.4 ± 14.2^c	288.4 ± 7.5^c	266.6 ± 17.6^c	
% 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 ± 2.78^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 two-way ANOVA test followed by Tukey's post hoc test. Values with different superscript letters represent significant differences ($P < 0.05$). $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

Effect of bee venom using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on levels of insulin and lipid profile

Table 7 displays the results of plasma insulin and lipid profile levels of all studied groups. For diabetic hyperlipidemic rats, a significant elevation in triacylglycerol, total cholesterol, LDL-C, and VLDL-C and a significant reduction ($P < 0.0001$) in HDL-C and plasma insulin levels were observed compared to the negative control. Treatment with bee venom using two doses (0.5 and 1.23 mg/kg) and a combination of metformin and atorvastatin significantly ameliorated ($P < 0.0001$) the undesirable changes in insulin and lipid profile compared to the positive control group.

Effect of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on serum CK-MB, LDH, and TnI

Table 8 shows the results of serum creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) activities and troponin I (TnI) for all studied groups. CK-MB and LDH activities were significantly elevated in the positive control as compared to the negative control group ($P < 0.0001$), while their activity was significantly reduced in bee venom-treated groups (0.5 and 1.23 mg/kg) and metformin and atorvastatin-treated group when compared to the positive control group ($P < 0.0001$). Concentration of serum troponin I (TnI) was significantly increased in the positive control when compared to the negative control group ($P < 0.0001$), whereas its level was significantly reduced ($P < 0.0001$) in bee venom-treated groups (0.5 and 1.23 mg/kg) and metformin and atorvastatin-treated group when compared to the positive control group.

Effect of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on TAC, MDA, and NF-$\kappa\beta$ levels in heart tissue

Total antioxidant capacity (TAC), malondialdehyde (MDA), and nuclear factor-kappa-β (NF-$\kappa\beta$) levels in cardiac tissue of all studied groups are summarized in Table 9. TAC was significantly decreased, whereas MDA and NF-$\kappa\beta$ were markedly increased in the positive control group when compared to the negative control ($P < 0.0001$). Compared to the positive control, the undesired elevations in MDA, NF-$\kappa\beta$, and decrease in TAC were substantially

Table 7. Effect of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on levels of insulin and lipid profile.

Groups	Negative control	Positive control	Bee venom-treated group (0.5 mg/kg)	Bee venom-treated group (1.23 mg/kg)	Metformin- and atorvastatin-treated group	*P* value
Insulin (pg/mL)	126 ± 1.16^c	57.9 ± 1.32	82.7 ± 2.03^c	121.6 ± 0.96^c	123.1 ± 1.14^c	<0.0001
% change	———————	−54.05%	42.83%	110.01%	112.61%	
Triacylglycerol (mg/dL)	82.8 ± 1.75^c	227.7 ± 4.3	158.5 ± 2.3^c	120.7 ± 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	104.6 ± 1.52^c	96.5 ± 1.15^c	
% change	———————	179.47%	−29.15%	−55.6%	−59%	
LDL-C (mg/dL)	40.14 ± 1.32^c	178.24 ± 2.5	113.6 ± 2.32^c	54.96 ± 1.63^c	46.1 ± 1.13^c	
% change	———————	344.04%	−36.26%	−69.16%	−74.13%	
VLDL-C (mg/dL)	16.56 ± 0.35^c	45.66 ± 0.91	31.7 ± 0.46^c	24.14 ± 0.34^c	21.6 ± 0.28^c	
% change	———————	175.72%	−30.57%	−47.13%	−52.69%	
HDL-C (mg/dL)	27.6 ± 0.47^c	11.7 ± 0.34	21.6 ± 0.45^c	25.5 ± 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 two-way ANOVA test followed by Tukey's post hoc test. Values with different superscript letters represent significant differences ($P < 0.05$). $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

Table 8. Effect of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on serum CK-MB, LDH, and *TnI*.

Groups	Negative control	Positive control	Bee venom-treated group (0.5 mg/kg)	Bee venom-treated group (1.23 mg/kg)	Metformin- and atorvastatin -treated group	*P* value
CK-MB(U/L)	210.8 ± 2.48^c	527.4 ± 28.39	324.9 ± 15.17^c	252.3 ± 4.95^c	240.8 ± 6.75^c	<0.0001
% change	———	150.2%	−38.39%	−52.16%	−54.34%	
LDH (U/L)	498.4 ± 2.65^c	1188.5 ± 42.57	711 ± 39.9^c	570.7 ± 10.45^c	567.2 ± 22.7^c	
% change	———	138.46%	−40.17%	−51.95%	−52.27%	
TnI (ng/mL)	0.081 ± 0.004^c	0.167 ± 0.006	0.11 ± 0.005^c	0.089 ± 0.007^c	0.092 ± 0.003^c	
% change	———	106.17%	−34.13%	−46.7%	−44.9%	

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 ($P < 0.05$). $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ compared to positive control group. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

Table 9. Effect of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on levels of TAC, MDA, and NF-$\kappa\beta$ in heart tissue.

Groups	Negative control	Positive control	Bee venom-treated group (0.5 mg/kg)	Bee venom-treated group (1.23 mg/kg)	Metformin- and atorvastatin -treated group	*P* value
TAC (mM/L)	0.95 ± 0.03^{c}	0.62 ± 0.03	0.75 ± 0.014^{b}	0.81 ± 0.01^{c}	0.89 ± 0.02^{c}	<0.0001
% change	————	−34.7%	20.96%	30.6%	43.54%	
MDA (nmol/g tissue)	79.8 ± 1.03^{c}	117.9 ± 2.7	101.4 ± 1.29^{c}	94.5 ± 1.44^{c}	92 ± 1.1^{c}	
% change	————	47.74%	−13.99%	−19.84%	−21.96%	
NF-$\kappa\beta$ (pg/mL)	90.1 ± 1.47^{c}	238.3 ± 3.3	174.4 ± 3.08^{c}	158.3 ± 1.9^{c}	133.5 ± 1.4^{c}	
% change	———	164.5%	−26.81%	−33.57%	−43.97%	

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. % change of positive control was calculated according to negative control. % change of all treated groups was calculated according to positive control.

improved after the administration of bee venom (either 0.5 or 1.23 mg/kg) and the combined therapy of metformin and atorvastatin ($P < 0.0001$).

Effect of bee venom using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on mRNA relative expression of vascular cell adhesion molecule-1 (VCAM-1) and galectin-3 in heart tissue

mRNA relative expression VCAM-1 and galectin-3 in heart tissue in all studied groups are demonstrated in Figure 1. The cardiac expression of VCAM-1 and galectin-3 genes of the positive control group was significantly higher than that of the negative control group ($P < 0.0001$). Overexpression of both VCAM-1 and galectin-3 was markedly declined after administration of bee venom (either 0.5 or 1.23 mg/kg) and the combined therapy of metformin and atorvastatin when compared to the positive control ($P < 0.0001$).

Histopathological examination

Histopathological results regarding cardiomyocytes in heart sections revealed normal myocardial muscles striation and nucleation in the negative control group (Figure 2(a)). Conversely, the positive group experienced myocarditis, hyalinization of myocytes and leucocytic cells infiltration (Figure 2(b)). Heart from bee venom-treated group (0.5 mg/kg) displayed a picture nearly resembling positive one as they existed with inflammation and hyalinization (Figure 2(c)). Unlike bee-venom-treated group (1.23 mg/kg) (Figure 2(d)), metformin- and atorvastatin-treated group (Figure 2(e)) exhibited a normal myocardial muscles striation and nucleation identical to negative control group.

Regarding the blood vasculature in heart sections, outcomes revealed a normal histological structure of blood vessels in negative control group (Figure 3(a)) and bee venom-treated group (1.23 mg/kg) (Figure 3(d)). On the other hand, results from positive group (Figure 3(b)) and bee venom-treated group (0.5 mg/kg) (Figure 3(c)) confirmed a prominent congestion to vessels distended with blood and also to myocardial muscles near it. Metformin- and atorvastatin-treated group revealed blood congestion in some vessels and others seemed normally (Figure 3(e)).

Effect of bee venom using both doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on area percentage (area %) of abnormal myocytes

Quantitative analysis of area percentage (Area %) of abnormal myocytes confirmed the photographic sections and resulted in a significant variation between the positive control group and all studied groups ($P < 0.0001$), while a non-significant difference was recorded between the negative control group and bee venom-treated group (1.23 mg/kg), as well as for the group treated with metformin and atorvastatin as shown in Figure 4.

Discussion

Hyperglycemia and increased oxidative stress are associated with a higher risk of cardiovascular disease in diabetics.[48] Hyperlipidemia is a widespread metabolic disorder characterized by high levels of LDL-C and triacylglycerol, which play a critical role in the pathogenesis of cardiovascular disease.[49]

Recently, great attention has been devoted to the treatment of different diseases using natural products. Bee venom is a natural substance secreted by honeybee queens and workers to defend themselves against invaders.[50] Venoms are toxic substances; however, they are characterized by therapeutic benefits.[51] The hypoglycemic activity of bee venom has been demonstrated in animal studies.[13,25,52] In obese experimental animals, bee venom reduces lipid deposition and adipose tissue inflammation is induced by a high-fat diet.[14]

This research aims to investigate the bee venom effects on cardiac dysfunction compared to the combined therapy of metformin and atorvastatin in diabetic hyperlipidemic rats.

The toxicity was estimated by intraperitoneal administration of Carniolan bee venom (*Apis mellifera carnica*) in rats to determine the median lethal dose (LD_{50}). According to the results, LD_{50} was 12.3 mg/kg. These findings were near to those obtained in a previous study,[53] reporting the LD_{50} value of Carniolan bee venom

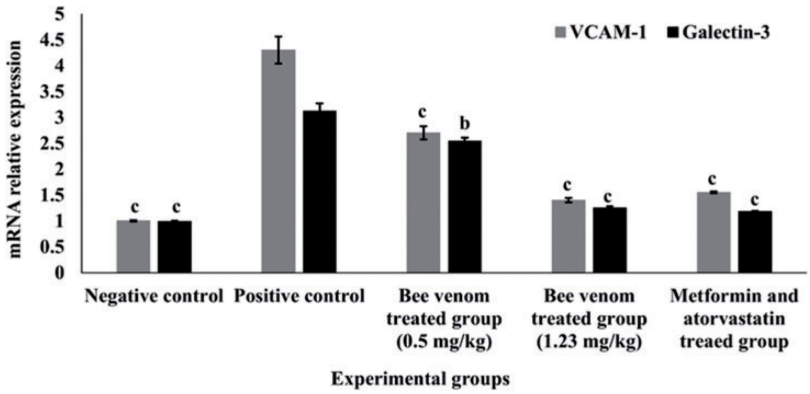

Figure 1. Effect of bee venom using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on mRNA relative expression of VCAM-1 and galectin-3 in heart tissue of all studied groups. Values are expressed in (mean ± SEM, $n = 6$).Statistical analysis was performed by two-way 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.

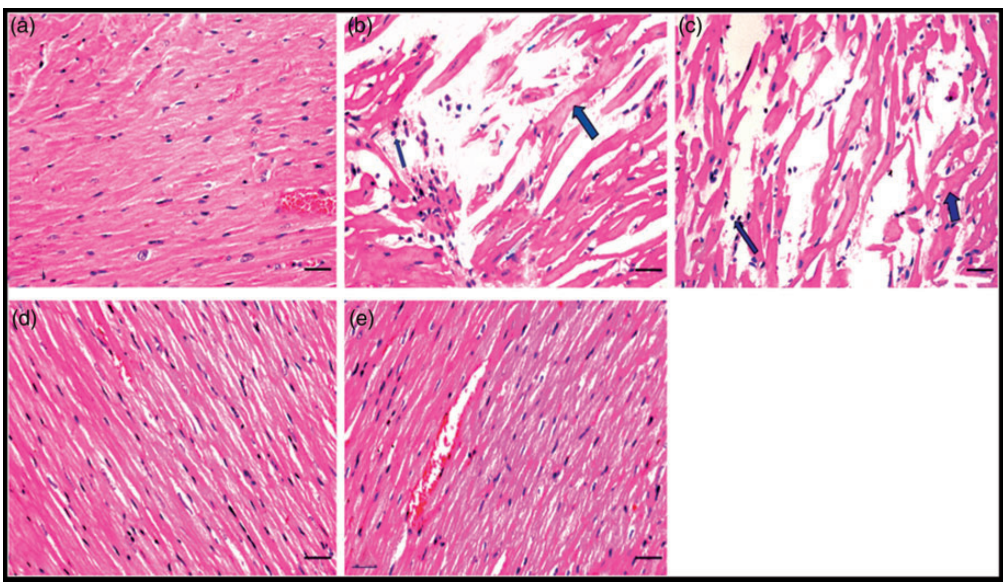

Figure 2. Photomicrographs of cardiomyocytes in heart sections of all studied groups: (a) Heart section from negative control group showed normal myocardial muscles striation and nucleation. (b) Heart section from positive group presented myocarditis, the hyalinized myocytes (thick arrow) and leucocytic cells infiltration (thin arrow). (c) Heart section from bee venom-treated group (0.5 mg/kg) displayed hyalinized myocytes (thick arrow) and leucocytic cells infiltration (thin arrow). Histological heart section from (d) bee venom-treated group (1.23 mg/kg) and (e) metformin- and atorvastatin-treated group exhibited the normal structure of myocardial muscles (H&E staining, scale bar = 100 μM). (A color version of this figure is available in the online journal.).

(8.47 mg/kg) when injected intravenously into mice. In an earlier study, the LD_{50} of Sweet Bee Venom in rats was over 30 mg/kg through single-dose toxicity tests.[54] Melittin and phospholipase A2 resulted in the toxicity of bee venoms that act synergistically.[55] The venom of all Apis species is similar in quality and composition. However, the differences in LD_{50} values can be attributed to the geographical distribution of different Apis mellifera species[56] and variations in their production and toxicity depending on their size and physiological differences.[57,58]

Streptozotocin-induced diabetes mellitus causes a decline in the bodyweight due to structural protein degradation and carbohydrate metabolism disturbance related to increased blood glucose and deficiency or no insulin secretion.[59]

Our data illustrated a significant decline in the bodyweight of the positive control group when compared to the negative control group. These findings support a previous study that found that insulin deficiency inhibits the body's ability to transfer glucose from the bloodstream to cells where it can be utilized. When this occurs, the body starts to consume fat and muscles for energy, resulting in decreased total bodyweight.[60] At the fourth week of the experimental trial, groups treated with bee venom using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) showed a significant elevation in bodyweight compared to the positive control group. Administration of bee venom improved the bodyweight, which may be due to improved glycemic control and its ability to elevate insulin secretion.[25]

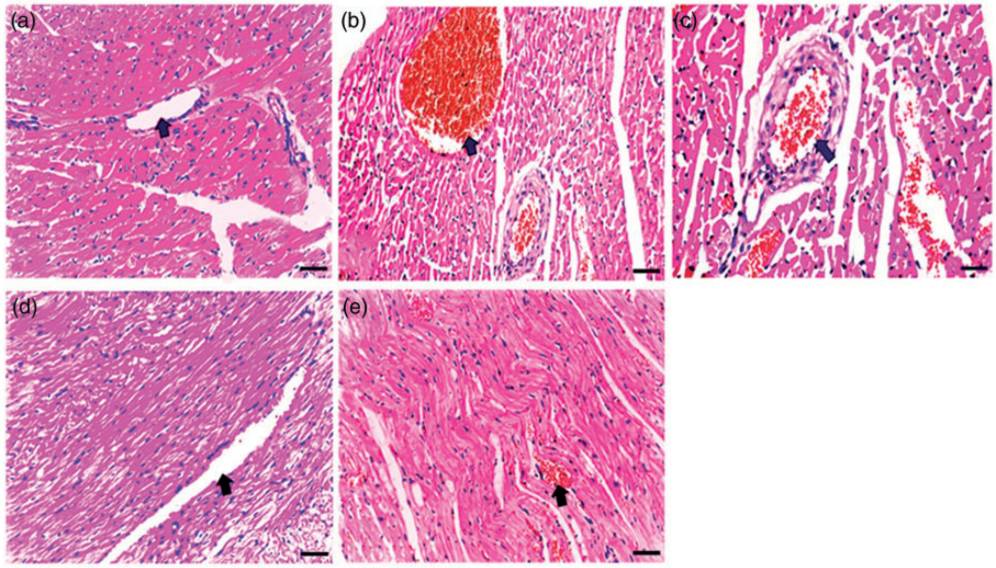

Figure 3. Photomicrographs of blood vasculature in heart sections of all studied groups: (a) Heart section from negative control group displayed normal blood vessels (arrow). Sections from (b) positive group and (c) bee venom-treated group (0.5 mg/kg) exhibited congested vessels distended with blood (arrows). (d) Blood vessels of heart section from bee venom-treated group (1.23 mg/kg) existed in a normal structure (arrow). (e) Heart section from metformin- and atorvastatin-treated group showed blood congestion in some vessels (arrow). (H&E staining, scale bar = 100 μM). (A color version of this figure is available in the online journal.).

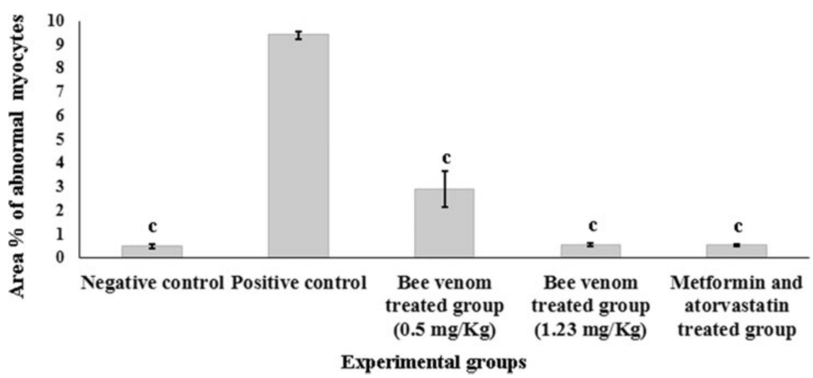

Figure 4. Effect of bee venom using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) on area % of abnormal myocytes in H&E stained heart sections of all studied groups. Values are expressed in (mean ± SEM, $n = 6$). Statistical analysis was performed by two-way ANOVA test 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.

In streptozotocin-diabetic rats, the decline in blood glucose levels can contribute weight gain.[61]

Streptozotocin (STZ) causes selective islet β-cell damage correlated with decreased β-cell function and viability, which eventually leads to an elevation in blood glucose and a reduction in insulin secretion.[62]

The findings indicated that the positive control group had a significant increase in fasting blood glucose levels and a significant decrease in plasma insulin levels when compared to the negative control group. The current findings are in agreement with those of an earlier study,[63] which found that the high levels of glucose and the decreased levels of insulin in diabetic rats with hyperlipidemia are because of STZ's cytotoxic influence, causing the degradation of β cells and a decline in insulin secretion in the positive control group compared to the negative control group. Meanwhile, bee venom-treated groups using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) exhibited a significant decline in the levels of fasting blood glucose and a substantial increase in plasma insulin levels compared to the positive control group. These results are in line with other studies[13,25,52] that confirmed the hypoglycemic activity of bee venom and its ability to raise insulin secretion. These glucose and insulin level improvements may be because of melittin and phospholipase-A2, which are active compounds in bee venom. They reduce the inflammation of the Langerhans islets and induce them to secrete insulin.[64,65] Bee venom also blocks pro-inflammatory cytokines and free radicals production, leading to beta-cell apoptosis.[66] Furthermore, melittin can depolarize β-cells plasma membranes that results in opening Ca^{2+} channels, allowing a significant amount of Ca^{2+} to enter β-cells and stimulating insulin secretion.[67]

Hyperglycemia and insulin action defects may contribute to lipid metabolism abnormalities and dyslipidemia in diabetics.[68] HFD consumption leads to increased serum total cholesterol triacylglycerol and LDL-C and causes hyperlipidemia.[69] Increased fatty acid oxidation and/or hepatic synthesis of VLDL-C, as well as the release of stored fatty acids from adipose tissues without a corresponding increase in the rate of clearance from the blood using lipoprotein lipase, may result in hyperlipidemia.[70]

The results demonstrated a significant rise in total cholesterol, triacylglycerol l, LDL-C, and VLDL-C levels and a marked decline in HDL-C levels in the positive control group compared to the negative control group at the end of the experimental period. These findings are consistent with those in other studies,[71,72] which reported that the levels of triacylglycerol, total cholesterol, LDL-C, and VLDL-C were significantly increased, and the HDL-C levels were declined considerably in diabetic hyperlipidemic rats because of the damaging effect of streptozotocin on β-cells and HFD consumption that results in reduced insulin secretion, hyperglycemia, and hyperlipidemia, while the groups treated with bee venom using both doses (0.5 and 1.23 mg/kg) and the combined therapy (metformin and atorvastatin) showed a significant decline in total cholesterol, triacylglycerol, LDL-C, VLDL-C levels, and a significant elevation in HDL-C levels in comparison to the positive control group at the end of the experimental period. The results are supported by other studies,[73,74] which revealed the ability of bee venom to alleviate lipid profile abnormalities through increasing insulin secretion and sensitivity in experimental animal models. Phospholipase A2 activity in bee venom is essential for lowering total cholesterol, triacylglycerol (TAG) and LDL-C, and regulating the lipid profile. Phospholipase A2 partially lyses the cell membrane of fatty tissues, leading to an increase in lipid uptake and glucose transport into adipocytes and raises the affinity of insulin molecules.[75]

Hyperglycemia, hyperlipidemia, and insulin resistance may result in raised cellular oxidative stress in the diabetic state. Reactive oxygen species (ROS) cause structural degradation of the cell membrane and lipid peroxidation, resulting in myocardial enzyme leakage. It is necessary to inhibit ROS generation to relieve myocardial reperfusion injury.[76]

Our results illustrated a significant elevation ($P < 0.0001$) in the activities of CK-MB and LDH of the positive control when compared to the negative control. In addition, they are compatible with other studies,[77,78] which found that the activities of CK-MB and LDH were raised in STZ-diabetic rats, likely due to disruption of myocardial structure induced by reactive oxygen species, while groups treated with bee venom using both doses (0.5 and 1.23 mg/kg) and the combined therapy (metformin and atorvastatin) showed a significant reduction ($P < 0.0001$) in CK-MB and LDH activities when compared to the positive control. These results are in accordance with Shaaban and Hamza,[79] who stated that treatment with bee venom resulted in a reduction in CK-MB and LDH activities compared to the positive control. The ameliorating effect of bee venom on CK-MB and LDH activities could be because of its antioxidant effect by suppressing the formation of free radicals, scavenging free radicals, preventing lipid peroxidation of membranes, and maintaining membrane integrity, thus limiting the outflow of these enzymes.[80]

The results showed a significant increase in serum troponin I in the positive control group compared to the negative control group. These results are in line with those in other studies,[81,82] which reported that the levels of troponin I were significantly increased in diabetic rats due to prolonged hyperglycemic condition, oxidative stress, and therefore the cardiomyocytes are damaged and troponin I is highly expressed which can be detected by various measures to indicate the myocardial injury. While groups treated with bee venom using both doses (0.5 and 1.23 mg/kg) and the combined therapy (metformin and atorvastatin) showed a significant reduction in the concentration of troponin I when compared to the positive control. These results are in accordance with Shaaban and Hamz,a[79] who stated that treatment with bee venom resulted in a reduction in troponin I levels compared to the positive control group, demonstrating the myocardial protection of bee venom because of the antioxidant effect of bee venom.

Numerous multiple causes have been proposed to understand why oxidative stress is raised, and these causes fall into two basic categories: raised reactive oxygen species (ROS) production and reduced antioxidant defense mechanisms.[83]

The results reported a significant decline in total antioxidant capacity (TAC) in heart tissue of the positive control compared to the negative control. These findings are consistent with those of a previous study,[84] which stated that the levels of total antioxidant capacity were declined in the hearts of diabetic rats because of adverse consequences of diabetes mellitus. Groups treated with bee venom using two doses (0.5 and 1.23 mg/kg) and the combined therapy (metformin and atorvastatin) showed a significant elevation in levels of total antioxidant capacity in comparison to the positive control group. These findings are in line with those of a previous study,[13] which reported that total antioxidant capacity levels in in groups treated with bee venom using two doses low-dose (1 mg/kg) and high-dose (2 mg/kg) were increased compared to the positive control because of the antioxidant effect bee venom and its ability to suppress the production of free radicals and scavenge them.

Malondialdehyde is one of the end products of lipid peroxidation process and can be utilized as a biomarker to assess oxidative stress levels. It is elevated in diseases-induced oxidative stress, including diabetes mellitus.[85]

Our results stated that the levels of malondialdehyde (MDA) in heart tissue of the positive control were significantly increased compared to the negative control. These data are in line with an earlier study,[86] which reported that the levels of malondialdehyde were increased in the heart of diabetic rats because of damage to the antioxidant defense system and a boost in lipid peroxidation. While groups treated with bee venom using both doses (0.5 and 1.23 mg/kg) and the combined therapy (metformin and atorvastatin) showed a significant decline in malondialdehyde levels in comparison to the positive control. These results follow a previous study,[79] which reported that the treatment with bee venom leads to a reduction in the malondialdehyde levels in heart tissue compared to positive control because of the anti-oxidative activity of bee venom.

Nuclear factor-kappa β (NF-$\kappa\beta$) is a critical transcriptional factor that is prone to oxidative stress induced by changes in glucose levels within the cell.[87] Activated NF-$\kappa\beta$ acts as an inducer of many inflammatory-related genes, vascular cell adhesion molecules (VCAM-1), tumor necrosis factor-alpha (TNF-α), and cytokines like interleukin-1 beta (IL-1β), IL-6, aggravating the vascular endothelial dysfunction and tissue injury.[88]

Our results reported that the levels of nuclear factor-kappa β (NF-$\kappa\beta$) in heart tissue in the positive control are significantly elevated compared to the negative control. These findings are supported by other studies,[89,90] which stated that the levels of nuclear factor-kappa β were elevated in the positive control in comparison to the negative control because hyperglycemia and oxidative stress are associated with the activation of NF-$\kappa\beta$ in the heart tissue of diabetic rats. Furthermore, activated NF-$\kappa\beta$ contributes to an elevated inflammatory response to cardiac tissue injury in diabetic rats. Groups treated with bee venom using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) showed a significant decline in NF-$\kappa\beta$ levels in heart tissue compared to the positive control. These results are compatible with those obtained in an earlier study,[91] demonstrating that bee venom exhibits an anti-inflammatory activity that is attributed to direct inhibition of the inflammatory response by inhibiting NF-$\kappa\beta$ activation.

Vascular cell adhesion molecule-1 (VCAM-1) is one of the cell-surface proteins family that helps cells adhere to each other and the extracellular matrix. Several factors induce VCAM-1 expression on the cell surface, including hyperglycemia, LDL-C, pro-inflammatory cytokines, and oxidative stress, leading to initiation of several pathways that contributed to VCAM-1 activation, including interferon regulatory factor-1, NF-$\kappa\beta$, and activating protein-1 (AP-1).[92] Once VCAM-1 expressed on the endothelial cell membrane, it binds to multiple ligands on leucocytes, including $\alpha4\beta1$ integrin, $\alpha4\beta7$ integrin, and galectin-3,[93,94] causing cytoskeletal remodeling and weakening of endothelial cell intercellular junctions, facilitating leukocyte transendothelial migration, which is an important step in the development of atherosclerosis.[95]

Our results revealed that the relative mRNA expression of VCAM-1 was significantly increased in the positive control when compared to the negative control. These findings are in agreement with a previous study,[96] indicating that after STZ injection, severe inflammation was observed in diabetic rats' cardiac tissue, which was initiated by the activation of the NF-$\kappa\beta$ signaling pathway, leading to an extreme expression of VCAM-1 identified by its mRNA level compared to the negative control group. Groups treated with bee venom using two doses (0.5 and 1.23 mg/kg) and the combined therapy (metformin and atorvastatin) showed a significant decline in relative mRNA expression of VCAM-1 in the heart tissue compared to positive control. These data are consistent with those of a previous research,[97] which demonstrated that treatment with bee venom resulted in a reduction in vascular inflammation and downregulation of VCAM-1 expression in the heart of atherosclerotic mice, implying that bee venom has anti-inflammatory and anti-atherogenic effects by suppressing the activity of pro-inflammatory cytokines like TNF-α and endothelial adhesion molecules such as VCAM-1 that contribute to atherosclerotic lesion formation.

Galectin-3 belongs to the β-galactoside-binding lectin family and is widely distributed in the heart, lung, brain, blood vessels, and visceral adipose tissue. Galectin-3 levels are higher in diabetes patients and obesity, which have been linked to problems with glucose homeostasis.[98] Overexpression of cardiac galectin-3 leads to interstitial fibrosis, myocardial macrophage infiltration, and heart dysfunction, and these effects are mediated by the activation of extracellular signal-regulated kinase 1/2 (ERK1/2), one of the best-characterized members of the mitogen-activated protein kinase (MAPK) family.[99] The activation of MAPK/ERK1/2 signaling pathway by pro-inflammatory mediators leads to activator protein-1 (AP-1) and transcription factor NF-κB, resulting in upregulation of galectin-3 expression.[100]

The results indicate a significant rise in relative galectin-3's mRNA expression in the positive control group compared to the negative control group. These results are in accordance with an earlier study,[101] reporting that the galectin-3 levels were increased in the left ventricular (LV) tissue of diabetic mice. Furthermore, it has been demonstrated that the relative mRNA expression of galectin-3 expression was upregulated in the hearts of rats fed a high-fat diet compared to those fed a standard diet.[102] The observed elevation in relative gene expression of galectin-3 may be related to heart impairment, suggesting that diabetes mellitus and a high-fat diet cause vigorous cardiomyocyte damage. However, groups treated with bee venom using two doses (0.5 and 1.23 mg/kg) and combined therapy (metformin and atorvastatin) showed a significant decline in relative galectin-3's mRNA expression in the heart tissue compared to the positive control. These findings are compatible with a previous study[103,] stating that bee venom has anti-inflammatory activity and can inhibit the ERK1/2 pathway, leading to negative regulation of AP-1 and NF-κB that are two transcriptional factors involved in the regulation of galectin-3 expression.

Histopathological studies of cadiomyocytes and blood vasculature in heart sections revealed that there was myocarditis, hyalinized myocytes, leucocytic cell infiltration, and congested vessels distended with blood in the positive control rats, while the negative control rats showed normal myocardial muscle striation, nucleation and blood vessels. These results are in line with a previous study,[104] which reported an obvious inflammation, disarranged cardiac myocytes, congestion and intracytoplasmic vacuoles in the heart sections of the diabetic group. So, these results had illustrated the increased levels of CK-MB, LDH, TnI, and NF-κB. I. Bee venom-treated group (0.5 mg/kg) displayed a picture nearly resembling positive control as they existed with inflammation, hyalinization, and congested blood vessels, but treatment with bee venom (1.23 mg/kg) and combined therapy (metformin and atorvastatin) restored normal heart muscles, normal myocardial muscles striation, and nucleation in contrast to the positive control, but bee venom-treated group (1.23 mg/kg) showed a normal histological structure of blood vessels. Meanwhile metformin- and atorvastatin-treated group revealed blood congestion in some vessels and others seemed normally. These findings are consistent with those of a previous study,[105] which demonstrated the mitochondrial ultrastructural integrity and preserved myofilament in the heart tissue of the bee venom-treated group. Also in another study,[106] it was reported that bee venom treatment successfully improved the abnormalities observed in heart sections compared to positive control group. The low-dose bee venom-treated group exhibited slight capillary telangiectasia and congestion, arranged myocardial fibers and a few small round vacuoles were observed in the myocardium, but medium and high dose of bee venom performed better than the low dose; myocardial fibers were abundant, and the arrangement of the fibers was close without other obvious abnormalities. The cardioprotective effects of bee venom can be ascribed to the reduction of oxidative stress and inflammation through inhibition production of reactive oxygen spices and inflammatory mediators due to its antioxidant and anti-inflammatory activities.[15,16,106]

A quantification analysis of the area % abnormal cardiomyocytes was also performed, revealing a significant difference between the positive control and all treated groups and a non-significant difference between the negative control and all treated groups. These results demonstrate the potential effect of bee venom-treated group (1.23 mg/kg) plus metformin and atorvastatin treated group. These results are in accordance with a previous study,[106] which confirmed the cardioprotctive effects of bee venom.

Conclusions

Based on the results obtained, the bee venom (BV) administration has a therapeutic effect on biochemical, mRNA expression, and histopathological abnormalities in diabetic hyperlipidemic rats by promoting insulin secretion, glucose, and lipid uptake in adipose tissue through its hypoglycemic and hypolipidemic activities. It can suppress upregulation of VCAM-1 and galactin-3 genes expression in the heart, which is associated with cardiac disease improvement through attenuating oxidative stress and concentrations of nuclear factor-kappa-β (NF-$\kappa\beta$) due to its antioxidantand anti-inflammatory effects. Bee venom can attenuate cardiac dysfunction in diabetic hyperlipidemic rats in a dose-dependent manner. Therapeutic dose (1.23 mg/kg) of bee venom and combined therapy (metformin and atorvastatin) roughly correct biochemical, mRNA expression, and histopathological abnormalities in comparison to the therapeutic dose (0.5 mg/kg) of bee venom.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, the interpretation of the studies, data analysis and review of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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