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Assessment of bee venom therapy in animal model of statin-induced myopathy

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Abstract

Background: Statin-induced myopathy is the most common adverse effect of statins. Bee venom provides a potential mean of controlling immune responses and inflammatory reactions; the proposed mechanisms for statin-induced myopathy.

Objective: The present study aimed at clarification of the role of the bee venom in prevention of statin-induced myopathy.

Materials and methods: It was carried out on 30 Sprague-Dawley female rats. Rats were randomly classified into 3 groups: control group, statin group which received statins for 2 weeks, and venom group that was exposed to alternate day actual bee sting concurrent to statins administration for 2 weeks. Quantitative electromyography (QEMG) was performed as well as serum creatine kinase (CK) and cholesterol levels, in addition to in vitro muscle contractility tests.

Results: QEMG and contractility tests showed significant changes in the statin group compared to both control and venom groups. Serum cholesterol level decreased with increase in CK levels in the statin and venom groups compared to controls; however, the CK level was significantly lower in the venom group as compared to the statin group.

Conclusion: Bee venom therapy offers a simple and available means of prophylaxis against the myopathic effects induced by statins in animal model. However, it partly restricts the therapeutic effect of statins.

Keywords: Statin-induced myopathy, Bee venom, QEMG, Muscle contractility

Introduction

Drug-induced myopathy is a common adverse effect that has been reported with several drugs including steroids, alcohol, colchicine, azidothymidine, clofibrate, and cholesterol-lowering agents [1]. Statins are cholesterol-lowering agents that are widely used in treatment and prophylaxis of atherosclerotic cardiovascular diseases. Statin-associated muscle symptoms are the most important cause of discontinuation of treatment [2]. Their effect on skeletal muscles ranges from muscle fatigue and weakness to pain and fatal rhabdomyolysis [3]. Some individuals may even develop a form of statin-induced autoimmune necrotizing myopathy characterized by being persistent and progressive despite drug removal [4].

The bee venom is a natural toxin produced by the honey bees to protect themselves against their predators [5]. It is a mixture of bioactive molecules that have immunogenic and neurotoxic effects. Bee venom therapy is the use of live bee stings or injectable venom to treat various diseases [6].

Bee venom therapeutic effect can be attributed to its anti-inflammatory, anti-fibrotic, immunomodulatory, and anti-apoptotic effects [7]. A cascade of reactions is produced once the bee venom enters the human body. The exact mechanism of action is still unknown. The potential adverse effects of bee venom therapy range from trivial skin reactions to life-threatening severe immunological responses [8]. Evidence-based studies concerning bee venom therapy in medical practice are still insufficient [9].

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Electromyography and CK level are the most common screening tests for diagnosing muscle myopathies and remain the most important techniques for assessing the course of the disease [10].

The present study aimed at clarification of the role of the bee venom in control or prevention of statin-induced myopathy in animal model using quantitative electromyography (QEMG), CK level, and contractility test.

Materials and methods

This is an experimental, prospective, interventional study. Thirty Sprague-Dawley female rats weighing 100–120 g and 6–8 weeks old were used. They were placed in a room at 23 ± 1 °C on an alternating 12:12-h light–dark cycle and maintained on standard rat chow diet consisting of 12.5% lipids, 63.2% carbohydrate, and 24.3% protein, with free access to water [11]. The study also included natural honeybees *(Apismellifera L).* The mean age of the bees was about 15 days old (after metamorphosis).

The animals were kept at the experimental animal house of Physiology Department, Faculty of Medicine, Cairo University.

All experimental procedures were conducted according to Guidelines for Ethical Conduct in the Care and Use of Nonhuman Animals in Research as approved by Experimental Animal Ethics Committee (Medical Experimental Research Center, Faculty of Medicine, Cairo University) [12].

The rats were divided into 3 equal groups of 10 rats each: (a) *control group*, they were kept on standard rat chow diet only; (b) *statin group*, they received daily treatment with simvastatin (88 mg/kg/day) for 2 weeks to induce myopathy [13]; and (c) *bee venom group*, they received daily treatment with simvastatin (88 mg/kg/day) concomitant with being subjected to a single bee sting at the origin of rat tail every other day for 2 weeks [14].

The calculated dose of simvastatin was diluted with water so that the maximal volume of drug-containing suspension was 1 ml and was administered using oral gavage.

The honeybee was held from the wings by forceps to put the venom containing sac close to the skin at the origin of the rat tail (Fig. 1). The defensive instinct induced the honeybee to sting the skin. The single bee sting contains about 140 μg of the bee venom [15].

Quantitative electromyography (QEMG) was done at the end of 2 weeks of drug administration, using Dantec Keypoint EMG machine (by the Natus Medical Incorporated, Pleasanton, USA). QEMG was carried out, using concentric needle electrode, for the right quadriceps femoris and right gastrocnemius muscles. For each muscle, the needle was inserted in three different sites and then the rat's right hind limb was motivated to move. Interference pattern analysis was performed 20 times at each site, where the number of turns, amplitude, turn/amplitude (T/A) ratio, number of small segments (NSS), and activity were recorded.

Fig. 1 The bee stinging process at the root of the rat tail

At the end of the study, blood samples were collected from retro-orbital plexus of veins, for biochemical analysis (serum creatinine phosphokinase and serum cholesterol).

For in vitro assessment of contractile function, longitudinal incision was done over the left limb to expose the gastrocnemius muscle. Its tendon was cut from its bone attachment and tied with a silk suture, maintaining its attachment to the knee joint and nerve supply. It was immediately submersed in cooled oxygenated (95% O_2 and 5% CO_2) Krebs solution at pH 7.4. The silk suture tied to the tendon was tied from the other end to a wide range force transducer (MLT1030/D -310, AD Instruments, Castle Hill Australia). The muscle was stimulated by using platinum-plate electrodes (electronic square wave stimulator, England) placed in close apposition of the bundle. A direct isometric twitch response was elicited by stimulating the muscle supra-maximally with 0.2-Hz rectangular pulses of 0.5-ms duration and recorded with an isometric transducer. After 10 min of thermo equilibration, contractile activity was continuously monitored and recorded using a PowerLab data acquisition system (4/30 - ML866-AD Instruments, Castle Hill Australia) with an ML110 bridge bioelectric physiographic amplifier.

The tension developed (from the baseline to the peak), contraction time (from the onset of tension record to the peak), and half-relaxation time (from the peak to fall of tension to half of peak value) were recorded.

Statistical analysis

It was carried out using Statistical Package for Social Sciences, version 22 for Windows® (SPSS Inc., Chicago, IL, USA). Data were expressed in the form of mean and standard deviation. One-way ANOVA was used to compare between more than two groups. $P < 0.05$ was considered significant.

Results

Compared to the control group, the statin group showed myopathic EMG features in the form of significant increase in turns, T/A ratio, activity, and NSS and showed

a significant decrease in amplitude whereas the bee venom group showed no significant difference, compared to the control group (Table 1).

The statin group showed a significant increase in number of turns and T/A turn ratio and significant reduction in amplitude, compared to the control group, whereas the bee venom group showed no significant changes (Table 2).

There was a significant decrease of CK in the bee venom group compared to the statin group; however, it was still significantly higher than the control group. There was a significant increase in the serum cholesterol in the bee venom group compared to the statin group; however, they were still significantly lower than the control group (Table 3).

The statin group showed a significant increase in contraction time and half relaxation time together with a significant reduction in tension, compared to both control and bee venom groups (Table 4).

Discussion

Statins are the most effective and commonly used drug for treatment of hypercholesterolemia [16]. Statin-associated myopathy includes a broad spectrum of conditions that range from benign myalgia to more serious inflammation and rarely may lead to life-threatening rhabdomyolysis [3]. Dealing with such common side effect is mandatory due to a lack of any other approved drugs for treatment of hypercholesterolemia apart from Ezetimibe which is significantly much less effective [17].

Table 1 Interference pattern analysis parameters of the quadriceps femoris muscle

Variables	Control $n = 10$	Statin $n = 10$	Bee venom $n = 10$	*P* value	
Turns (no./epoch)	444.56 ± 203.51	654.0 ± 93.87	500.88 ± 127.46	P1	0.004*
				P2	0.405
				P3	0.029*
Amplitude (μV)	423.56 ± 99.08	283.70 ± 62.68	435.38 ± 143.34	P1	0.01*
				P2	0.817
				P3	0.006*
Turn/amplitude (T/A) ratio	1.104 ± 0.306	1.997 ± 0.74	1.3 ± 0.543	P1	0.001*
				P2	0.439
				P3	0.1
NSS	210.22 ± 102.63	347.0 ± 83.99	268.75 ± 99.08	P1	0.003*
				P2	0.182
				P3	0.078
Activity (%)	19.44 ± 6.95	28.70 ± 6.67	21.38 ± 6.11	P1	0.004*
				P2	0.518
				P3	0.019*

NSS numbers of small segments, *P1* control group vs the statin group, *P2* control group vs bee venom group, *P3* statin group vs bee venom group.*Significant (*P* value ≤ 0.05)

Table 2 Interference pattern analysis parameters of the gastrocnemius muscle

Variable	Control $n = 10$	Statin $n = 10$	Bee venom $n = 10$	*P* value	
Turns (no./epoch)	434.89 ± 96.46	599.00 ± 164.28	471.43 ± 134.23	P1	0.011*
				P2	0.549
				P3	0.043*
Amplitude (μV)	410.22 ± 109.08	312.90 ± 47.70	382.86 ± 114.8	P1	0.031*
				P2	0.527
				P3	0.113
Turn/amplitude (T/A) ratio	1.104 ± 0.306	1.997 ± 0.74	1.3 ± 0.543	P1	0.001*
				P2	0.439
				P3	0.1
NSS	338.33 ± 137.74	388.60 ± 164.04	334.43 ± 104.26	P1	0.421
				P2	0.956
				P3	0.386
Activity (%)	32.67 ± 17.73	29.7 ± 12.07	29.86 ± 11.02	P1	0.64
				P2	0.66
				P3	0.98

NSS numbers of small segments, *P1* control group vs statin group, *P2* control group vs bee venom group, *P3* statin group vs bee venom group. *Significant (*P* value ≤ 0.05)

The therapeutic application of the bee venom has been used in traditional medicine to treat inflammatory and autoimmune diseases, such as rheumatoid arthritis, osteoarthritis, pain, and frozen shoulder [18]. The bee venom was also used for the treatment of different neurological conditions such as multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Alzheimer's, and Parkinson [19]. It was also tried recently as well for treatment of diabetic peripheral neuropathy and neuromuscular junction disease [20]. Yet, up to the authors' knowledge, there are no publications concerning the therapeutic use of the bee venom for myopathy neither in humans nor animals.

The present study was conducted to evaluate the effect of bee venom administration on the development of

Table 3 Serum creatinine phosphokinase and serum cholesterol in the studied groups

Variable	Control group ($n = 10$)	Statin group ($n = 10$)	Bee venom group ($n = 10$)	*P* value	
Serum creatinine phosphokinase (CK)	127.6 ± 3.97	532.75 ± 44.47	240.98 ± 17.12	P1	0.000*
				P2	0.000*
				P3	0.000*
Serum cholesterol	181.3 ± 10.38	84 ± 6.5	134.3 ± 6.34	P1	0.000*
				P2	0.000*
				P3	0.000*

P1 control group vs statin group, *P2* control group vs bee venom group, *P3* statin group vs bee venom group.*Significant (*P* value ≤ 0.05)

Table 4 In vitro assessment of contractile function of gastrocnemius

Variable	Control group (n = 10)	Statin group (n = 10)	Bee venom group (n = 10)		P value
Contraction time (msec)	37.91 ± 3.48	49.5 ± 4.1	40.94 ± 3.43	P1	0.000*
				P2	0.077
				P3	0.000*
Half relaxation time (msec)	52.98 ± 4.94	69.29 ± 5.74	53.09 ± 9.46	P1	0.000*
				P2	0.97
				P3	0.000*
Tension (N)	60.04 ± 5.22	26.61 ± 5.06	47.07 ± 3.75	P1	0.000*
				P2	0.000*
				P3	0.000*

P1 values compare the control group with the statin group, *P2* values compare the control group with the bee venom group, *P3* values compare the statin group with the bee venom group. *Significant (P value ≤ 0.05)

simvastatin-induced myopathy in rats. Rat model was chosen rather than humans as the use of bee venom therapy in humans require a specialized precaution in order to deal with any adverse effects of such therapy, ranging from simple allergy to severe fatal anaphylactic shock, which needs specialized life-saving measures. Its use in humans should only be used under the supervision of a qualified health care professional. Most experts recommend having an emergency sting kit available in case of allergic reaction including a syringe and a dose of epinephrine and antihistaminic tablets [21].

The protocol of statin administration to induce myopathy was adopted from previous studies; the work of Westwood and colleagues [22] utilized a dose of 80 mg/kg/day simvastatin to induce myopathy in rats and Mallinson and colleagues [13] who used the dose 88 mg/kg/day simvastatin for 12 days. As for bee stinging protocol, we applied the regimen of actual bee sting every other day for 2 weeks, as recommended by Sayed and colleagues [23], who applied this regimen to study the antimicrobial properties of the bee venom against staphylococcal infection. In another study assessing the anti-inflammatory effect of the bee venom in adjuvant-induced arthritis in rat, the whole bee venom was also administered subcutaneously every other day for 14 days [24].

Two muscles (a proximal as well as a distal one) were selected for QEMG assessment for better evaluation of the pattern of myopathy induced or prevented. Easy activation was another important factor for the choice of studied muscles. Authors settled on the hind limb muscles (quadriceps and gastrocnemius) as they fulfill the abovementioned criteria. As the gastrocnemius muscle is rather small in size and proper sampling for muscle quadrants was a little challenging, additional in vitro contractility tests were added to gastrocnemius for more precise evaluation.

Regarding CK, the present study reports that simvastatin induces CK elevation for more than 4 folds. These findings are in agreement with several other reports both in humans and animals [25–28]. Co-administration of the bee venom with simvastatin is found to partially reduce CK elevation to 2 folds only, which was still significantly high. This finding suggests that the bee venom can partially ameliorate the skeletal muscle cell damage induced by simvastatin.

The QEMG results showed significant changes especially in quadriceps muscle, being more proximal. The statin group showed significant changes in interference pattern analysis parameters, indicating skeletal myopathy, in the form of increase in the numbers of turns, turn/amplitude (T/A) ratio, NSS, and activity %, together with a decrease in the mean amplitude.

These findings are in line with that of Farouk and colleagues [29], who demonstrated that simvastatin in rats at different regimen of use induced myopathic EMG features which persisted even after simvastatin discontinuation. However, their study assessed gastrocnemius muscle only, analyzing just two parameters of the interference pattern (amplitude and duration) and did not document any spontaneous activity. Also, they assessed stem cell therapy for prevention of myopathy and reported its success. No other studies on rat model were found to compare the current study results with.

The myopathic picture can be due to random and diffuse degeneration as well as asynchronous firing of muscle fibers, reflected in the short duration, low-amplitude, and polyphasic shape of individual motor unit potentials. These changes in individual motor unit potentials influence the interference pattern, resulting in an increase in number of turns, a decrease in mean amplitude, an increase in turn/amplitude (T/A) ratio, and an increase in the number of small time intervals between turns [30, 31]. In literature, concerning subjects with myopathy, analysis of the interference pattern was more sensitive than the motor unit potential analysis [32]. Moreover, it was reported that myopathies are characterized by increased the numbers of turns and turn/amplitude (T/A) ratio, increased NSS, and decreased amplitude [33].

The present study demonstrated that simvastatin administration produces significant detrimental effects on skeletal muscles contractility as shown by the gastrocnemius simple muscle twitch parameters. Also, it was found that bee venom co-administration with simvastatin prevented these effects.

These findings agree with Simsek and colleagues [34] who reported that simvastatin administration resulted in a depression in the force-frequency curves in all muscles, indicating the impairment of muscle contractility.

The impairment of skeletal muscle contractility could be explained by the fact that simvastatin induces skeletal

muscle structural and functional alterations that are more profound in the fast-twitch than in the slow-twitch muscles. Moreover, the kinetics and functions of membrane ion channels were also affected, contributing to the statin-induced impairment of muscle contractility [35].

The lack of clinical signs of myopathy throughout a 2-week period of the study in spite the QEMG and CK results suggesting myopathy may be attributed to the short duration of the study which caused only subclinical myopathy.

The mechanism through which the bee venom might have partially prevented the simvastatin-induced myopathy is not clear as the proposed mechanisms of its induction of myopathy are multiple including inflammatory mitochondrial impairment and oxidative stress [35], passing through multifactorial induction of apoptosis [36] and autoimmune triggered myopathy [37], even genetic predisposition is hypothesized [38]. On the other hand, the bee venom has several components with different mechanisms of action and different therapeutic effects.

Anti-inflammatory effect and modulation of the activity of the immune system is the most relevant mechanism in the authors' opinion. It could be achieved through elevation of plasma cortisol [32], suppression of leukocyte migration and TNFα levels [39], reduction in cytokine production [14], and infiltration of CD4+ T cells at the site of inflammation [40]. Also, modulation of peripheral immune tolerance may contribute to the protective effect of the bee venom [41].

The bee venom has powerful antioxidant effect and apparently normalizes in structure of the mitochondria through prevention of micro-vasculopathy as documented histologically in a study conducted by Baher and Abo Zeid [20]. However, in the former study, normalization of microcirculation was attributed to reduction of hyperglycemia in diabetics, but in the current study, hypercholesterolemia reversal is the case.

The current results show significant reduction in cholesterol serum level in the bee venom group, though not as marked as in the statin group (still less than the control group). This finding suggests that the use of the bee venom reduces the cholesterol lowering effect of simvastatin but does not abolish it. Either reduction in simvastatin serum level or in affinity of drug to its receptor is suggested. Further studies including monitoring of simvastatin serum level and using other statins are recommended for better understanding and thus maximizing statin therapeutic effects during concurrent bee venom administration.

The current study offers a simple, cheap, easily available prophylaxis against the most common side effect of worldwide frequently used cholesterol-lowering agent (statins) with no need for its continuous dose adjustments or replacement or the use of sophisticated method as stem cell therapy offered in literature [29].

The use of the bee venom limits the action of statins on cholesterol level, so it may decrease the action of the drug. This may be the cause of decreased myopathic effect of the drug with the use of bee venom. So, further studies on a group of rats should be carried out with lower dose of statins, then compared with the higher dose group and the bee venom group. This is to be done before recommending the bee venom use in humans as it is must be done with caution because of its allergic reactions.

Paraffin blocks were prepared from muscle biopsy. Sections were cut at 2–4 microns, stained with hematoxylin and eosin and examined under light microscopy. No differences were revealed among groups, and unfortunately, further immune-histochemical studies were not available. In literature, variability in study design causes wide variation in the agreement between EMG and muscle biopsy. Constantinides et al. [42] investigated the diagnostic accuracy of muscle biopsy versus EMG in suspected myopathy and found that in some cases, there was a myopathic EMG with normal biopsy and explained it by the occurrence of normal biopsy in clinically healthy individuals showing mild myopathic findings so are less likely to yield positive biopsies. Also, Chang et al. [43] found nonspecific biopsy findings in some patients with myopathic EMG. Researchers investigating drug-induced myopathy relied upon EMG examination and CK only [29], which we relied on in the present study in addition to physiological studies. The safety of the bee venom as a therapeutic compound has been extensively studied. The potential adverse effects ranged from minor skin reactions to life-threatening immunological responses such as anaphylaxis [44]. According to different studies, 1 to 5% of the people worldwide are allergic to bees or other insects like wasps and hornets [45].

Abbreviations

ALS: Amyotrophic lateral sclerosis; CK: Creatine kinase; EMG: Electromyography; MS: Multiple sclerosis; NSS: Number of small segments; QEMG: Quantitative electromyography; T/A: Turn/amplitude; TNFα: Tumor necrosis factor alpha

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Authors' contributions

RA and EM designed the analyses. RA, EM, BBE, ASK, MG, and MM collected and analyzed the data. RA, EM, and ASK wrote the first draft of the manuscript. AAK, RA, EM, BBE, ASK, MG, and MM critically reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author [ASK]. The data are not publicly available due to data protection.

Ethics approval and consent to participate

This article contains studies with animals performed by the authors. The protocol of the study was discussed and approved by the Cairo University Institutional Animal Care and Use Committee (CU-IACUC), Medical Science Sector, the reference number CU/III/F/24/18.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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