Cardiac effects of bee venom in rats

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ABSTRACT

الأهداف: توضيح مدى تأثير زعاف النحل على كل من: خصائص القلب الكهربائية في الجسم، وخواص التقلص العضلي والميقاتية (معدل نبضات القلب) لعينات القلب المعزولة في المختبر، وتأثيره على استجابة القلب لتحفيز إطلاق الأدرينالين بواسطة الأيزوبروتيرينول.

الطريقة: أُجريت هذه الدراسة العشوائية والتي كانت من نوع حالة–شاهد في قسم الفسيولوجيا التابع لكلية الطب في جامعة عين شمس، القاهرة، مصر وذلك خلال الفترة من أبريل إلى يونيو 2010م. شملت الدراسة 22 جرذي أنثى من النوع ويستر، وقد تم تقسيمها عشوائياً إلى مجموعتين: مجموعة الحالة والتي تم علاجها بزعاف النحل بمقدار 20 ميكروغرام / كلغ تحت الجلد ولمدة 4 أيام، ومجموعة الشاهد. لقد خضعت الحيوانات المشاركة قبل قتلها لتخطيط القلب الكهربائي، وبعد ذلك قُتلت الجرذان وتم عزل عينات القلب في كلي المجموعتين، وتمت الاستعانة بتقنية لاجيندروف من أجل تحليل خواص عينات القلب داخل الجسم، ومدى استجابتها لإطلاق بيتا الأدرينالين. لقد أُخذت عينات من أنسجة القلب وذلك من أجل تقييم محتوى الكالسيوم في عضلة القلب، وعمل مجموعة من التحاليل للأنسجة.

النتائج: لقد كانت نتائج التخطيط الكهربائي للقلب طبيعية في مجموعة الحالة، كما أن زعاف النحل قد عمل على تحسين استجابة التوتر العضلي لتحفيز إطلاق بيتا الأدرينالين، بالإضافة إلى تحسين التقلص العضلي للقلب. وقد أشارت نتائج الدراسة إلى زيادة محتوى الكالسيوم في عضلة القلب في مجموعة الحالة. وأظهرت نتائج تحليل الأنسجة مدى الاحتفاظ باكتمال البنية المستدقة للخيط العضلي والميتوكندريا.

خاتمة: أثبتت هذه الدراسة مدى تأثير زعاف النحل على تحسين استجابة التقلص العضلي لمحفزات مستقبلات بيتا الأدرينالية، وقد قام زعاف النحل في نفس الوقت على حماية القلب من الأذى الناتج عن زيادة محتوى الكالسيوم.

Objectives: To elucidate the possible effects of bee venom (BV) on cardiac electrophysiological properties *in vivo*, the inotropic and chronotropic properties of the isolated hearts *in vitro*, and the cardiac responsiveness to progressive adrenergic stimulation by isoproterenol.

Methods: This randomized control study was conducted in the Physiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt, from April to June 2010. This work was carried out on 22 female Wistar rats. Rats were allocated into 2 groups; BV-treated group (rats were treated with BV in a dose of 20 µg/kg body weight, administered subcutaneously for 4 days), and the control group. Prior to sacrifice, the studied animals underwent electrocardiographic (ECG) assessments under anesthesia. Thereafter, isolated hearts were studied in a Langendorff preparation for their intrinsic properties, and their responses to ß-adrenergic stimulation. Following recovery, heart tissues were used for assessment of myocardial calcium content, and for histological examination.

Results: No abnormal ECG findings were observed in the BV-treated group. The BV treatment enhanced tension generation in the cardiac muscle in response to ß-adrenergic stimulation, and improved the inotropic cardiac reserve. Calcium content of the myocardial tissue of BV-treated group was significantly increased. Histological examination of the cardiac tissue of BV-treated group demonstrated preserved myofilament and mitochondrial ultrastructural integrity.

Conclusion: The BV enhanced the cardiac inotropic reserve to ß-receptor agonists. Meanwhile, BV protected the heart against calcium overload-induced injury.

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Bee venom (BV) has potential use in the medical field. The BV has been used for the treatment of inflammatory diseases, such as rheumatoid arthritis and relief of pain.[1] It has been demonstrated that treatment with BV provided a significant therapeutic effect in mice infected with intracerebral *Candidiasis*, and thereby could be effective in resistant life-threatening infections.[2] However, massive inoculation of BV can induce respiratory syndrome, liver injury, pancreatitis, skin necrosis, shock, hypertension, bleeding, thrombocytopenia, hemolysis, and rhabdomyolysis.[3] Several researchers have pointed out that BV induced cardiovascular abnormalities in animals.[4,5] Despite the cardiovascular effects that have been demonstrated by researchers, there is currently insufficient evidence on the cardiac effects of BV in the therapeutic dose used in the treatment of medical diseases. The systemic hemodynamic and neurohormonal factors in intact animals interfere with the identification of changes in intrinsic cardiac properties in such animals. The use of isolated Langendorff heart perfusion model *in vitro*, eliminates these influences. Thus, it reflects the intrinsic responsiveness of the heart. A major pathway to enhance cardiac performance is the increase in adrenergic responsiveness. As ß-adrenergic receptors regulate the rate and force of cardiac contraction and relaxation, they are of particular importance in the mammalian heart. Blunting of cardiac adrenergic responsiveness has been implicated in experimental models of cardiovascular diseases.[6] In view of the emerging promising therapeutic effect of BV, this study was conducted to probe the effects of BV therapy on intrinsic cardiac functions. The electrocardiographic (ECG) parameters, the baseline cardiac performance of the isolated hearts, as well as the chronotropic and inotropic reserve mechanisms, in response to *in vitro* cardiac stress by the ß-adrenoceptor agonist isoproterenol (ISO) were assessed. Furthermore, the effect of BV on the structure of the cardiac muscle was studied.

Methods. This randomized control study was conducted in the Physiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt, from April to June 2010, and was approved by the Faculty of Medicine Ain Shams University Research Ethics Committee, Cairo, Egypt. This work was carried out in 22 female Wistar rats weighing 120-150 gm, and age of 10-12 months. The rats were maintained under standard conditions of boarding. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health. Rats were allocated into 2 groups: a) BV-treated group - rats were treated with BV (BV [Apitoxin, Egyptian Organization for Biological Products and Vaccines [VACSERA], Cairo, Egypt])dissolved in saline, in a dose of 20 µg/kg body weight administered by subcutaneous injection (s.c.) daily for 4 days (n=10),[2] b) control group - consisted of rats injected with sterile saline (n=12). Blood picture, liver, and kidney function tests were assessed to exclude toxic effects of the applied dose of BV in this study.

Experimental procedures. On the fifth day following the first dose of BV injection, rats were weighed and injected intraperitoneally (i.p) with heparin sodium (1000 IU). Afterwards, the rats were anesthetized with thiopental sodium (40 mg/kg body weight; i.p). The ECG tracing was recorded using bipolar limb leads with recording speed of 25 mm/sec. Measurements were carried out for heart rate (HR), duration of P-R interval, and the observed Q-T interval ($Q\text{-}T_o$). Likewise, the observed Q-T interval was corrected for the effect of HR ($Q\text{-}T_c$). A midline incision was made, and the abdominal aorta was exposed and cannulated. Two blood samples were collected. One ml of blood was drawn into a tube containing ethylene diamine tetraacetic acid, and kept at room temperature for assessment of a complete blood picture within one hour from blood collection. Another blood sample was collected, and used for biochemical assessment. The hearts were removed using standard surgical techniques. The perfusion of isolated hearts was performed according to the method described previously.[7,8] The hearts were perfused in a Langendorff preparation, with retrograde perfusion under constant pressure (55 mm Hg) without recirculation. The perfusion medium used was the modified Krebs-Henseleit bicarbonate buffer (KHB) of pH 7.4, equilibrated with O_2 and CO_2 (95:5) at 37°C. The heart was left to stabilize for 10 minutes. A baseline recording was then obtained at a paper speed of 50 mm/sec, to determine the baseline heart beating rate (BR), beat per minute (bpm), developed peak tension (PT in g), time to peak tension (TPT in msec), rate of tension development (dT/dt in g/msec) and half relaxation time (½RT in msec). Myocardial flow rate (MFR) was determined by volumetric collection of the fluid passing out of the heart for 3 minutes, and was calculated relative to the left ventricular weight per minute (MFR /LV in ml/100mg/min). The ISO infusion was carried out through a catheter tube connected to an opening just above the aortic cannula. The ISO was dissolved and diluted with KHB, and infused using a Sega-355 infusion pump at sequential doses of $1.5x10^{-4}$, $2x10^{-4}$, $3x10^{-4}$, $4x10^{-4}$, and $6x10^{-4}$ mg per 3 minute. Each dose level of ISO was infused over 3 minutes, and then the recording was obtained for one minute at 50 mm/sec paper speed, and the flow was collected and measured. Maximal responses to ISO and delta changes, the difference between the maximal responses, and the basal

values were also calculated. Following heart perfusion, the hearts were washed with normal saline, blotted dry by filter paper, and were further cleaned from fat and fibrous tissue. The ventricles were then blotted, weighed and stored at -70°C until biochemical assay. The myocardial calcium content was determined according to the method of Oshiro et al.[9] The ventricular samples were placed into a platinum pot and heated to 200°C for one hour, and then into a muffle furnace of 700°C for one hour, afterwards they were allowed to cool to room temperature. Then, 10 ml of 50% nitric acid was added, and the samples were nitrified to clear and transparent solution under low electrical oven, and the calcium content was determined by atomic absorption spectrophotometry. Data were calculated relative to cardiac weight.

Electron microscopic study. Two rats from the control group were immediately sacrificed following perfusion without ISO stimulation to serve as histological control (untreated rats). Parts of the lower half of the left ventricle from the hearts of the studied experimental groups were fixed in 4% formolgluteraldehyde, dehydrated, and embedded in resin. Sections of 60 nm thickness were cut on copper grids and stained with uranyl acetate followed by lead citrate for examination using electron microscope.[10]

Biochemical determinations. Hematological parameters, renal and liver function parameters were assessed using standard laboratory methods (8 samples were discarded from the beginning because they were hemolyzed).

Data are expressed as means ± standard error of mean. Using the Statistical Package for Social Sciences version 10 (SPSS Inc, Chicago, IL, USA), unpaired t-test was carried out to determine differences between the BV-treated group and the control group. Paired t-tests were used to determine the difference in respective basal (preinfusion) values, and values at mentioned doses of ISO for different variables for both the BV-treated group and control groups. A confidence level of 95% was considered statistically significant. The level of significance was accepted as $p<0.05$.

Table 1 - Blood picture, liver, and kidney function parameters in bee venom (BV)-treated rats and the controls.

Variables	BV-treated rats (n=6)	Control (n=6)
Leukocytic count, $x10^3/\mu l$	4.45 ± 0.25	4.35 ± 0.26
Red blood cell count, $x10^6/\mu l$	6.79 ± 0.12	6.88 ± 0.01
Platelet count, $x10^3/\mu l$	738 ± 25.30	742 ± 30.68
SGOT, IU/dl	107.66 ± 1.9	107.16 ± 1.3
SGPT, IU/dl	25.16 ± 0.91	26.1 ± 0.87
BUN, mg/dl	44.16 ± 1.16	40.66 ± 1.35
Creatinine mg/dl	0.216 ± 0.008	0.203 ± 0.003

Data are expressed as mean ± standard error of mean. Significance was calculated by unpaired t-test at $p<0.05$ from the control group. SGOT - serum glutamic-oxaloacetic transaminase, SGPT - serum glutamic pyruvic transaminase, BUN - blood urea nitrogen

Results. A pilot study was carried out in 6 rats, which did not receive saline injection, used as negative controls, no significant difference in any of the electrophysiological parameters studied was observed as compared to the saline injected controls excluding positive and/or negative effects of the vehicle (saline).

Table 2 - Heart rate (HR), P-R interval, observed Q-T interval ($Q\text{-}T_0$), and corrected Q-T interval ($Q\text{-}T_C$ interval) of the 2 groups.

Groups	HR	P-R interval	$Q\text{-}T_0$ interval msec	$Q\text{-}T_C$ interval
Bee venom, n=10	437 ± 20.78	37 ± 0.001	77.78 ± 3.64	210.67 ± 11.19
Control, n=10	431 ± 13.27	38 ± 0.001	76.00 ± 2.67	206.20 ± 9.30

Data are expressed as means ± standard error of mean. Significance calculated by unpaired t-test at $p<0.05$ from the control group.

Table 3 - Effect of graded isoproterenol infusion on hearts isolated from bee venom (BV)-treated rats (n=10) compared to the controls (n=10).

Variables	Baseline values		Maximal responses		Delta changes	
	Bee venom	Control	Bee venom	Control	Bee venom	Control
BR, (bpm)	168 ± 12.24	175 ± 15.34	273 ± 16.97	249 ± 10.11	105 ± 18.23	73.7 ± 13.52
MFR, (ml /100mg/min)	3.29 ± 0.25	2.72 ± 0.22	2.90 ± 0.26	2.70 ± 0.19	-0.39 ± 0.33	-0.25 ± 0.24
dT/dt, (g/msec)	0.20 ± 0.02	0.25 ± 0.02	0.7 ± 0.07*	0.52 ± 0.06	0.49 ± 0.06*	0.27 ± 0.05
½RT, (msec)	61.11 ± 6.96	77 ± 5.17	38.89 ± 2.61	44 ± 2.61	-22.22 ± 5.72	-33 ± 5.39

Data are expressed as mean ± standard error of mean. *significance calculated by unpaired t-test at $p=0.04$ from the control group.
BR - heart beating rate, MFR - myocardial flow rate, dT/dt - tension generation, ½RT - half relaxation time

Blood picture, liver, and kidney function tests indicated non-toxic effects of the applied dose of BV in this study as shown in **Table 1**. *In vivo* HR, P-R interval, the observed Q-T interval ($Q\text{-}T_0$), as well as the corrected Q-T interval ($Q\text{-}T_C$) showed non-significant changes in the BV-treated group compared to the control group (**Table 2**).

In vitro studies on isolated perfused hearts with ISO infusion. The baseline values, the maximal responses to ISO and the delta changes, difference between the maximal and basal values for BR, MFR, as well as 1\2 RT showed non-significant difference between the hearts of the BV-treated group and their matching controls (**Table 3**). Although the baseline values for dT/dt showed non-significant difference between the hearts of the BV-treated group and the controls, the maximal responses to ISO and the delta changes, indicating enhancement of inotropic reserve were significantly higher in the hearts of the BV-treated group compared to controls (**Table 3**).

The ISO dose-response relationship (**Figure 1**). The ISO dose response curves of the studied cardiac function parameters showed a significant increase in the BR of the hearts isolated from the control group, as well as the hearts from the BV-treated group in response to all doses of ISO. The response of the hearts of the BV-treated group was significantly higher up to the last dose compared to their respective basal values. As regard MFR, the hearts isolated from the control group, as well as the hearts from the BV-treated group showed non-significant changes in response to the first 4 doses of ISO infusion. The hearts isolated from the control group showed a significant decrease in MFR in response to the last dose compared to their pre-infusion levels. Meanwhile, the hearts isolated from the BV-treated group showed a non-significant change in response to the last dose compared to their pre-infusion levels. Upon ISO infusion, the hearts of the control rats developed a significant increase in their dT/dt in response to all doses of ISO infusion except the last dose. The hearts of the BV-treated group showed a significant increase in response to all doses, and the response was significantly higher up to the last dose compared to their respective basal values. Upon ISO infusion, the hearts of the BV-treated group developed a significant enhancement in their ½RT in response to all doses of ISO infusion except the last dose. The hearts of the control rats showed a significant enhancement in response to all doses, and the response was significantly higher up to the last dose compared to their respective basal values.

Calcium assay in cardiac muscles. The hearts isolated from the BV-treated group showed significantly higher calcium content (870±17.54 µg/g LV) compared to the control group (17.54±0.34 µg/g LV). The significance was calculated using unpaired t-test (p=0.001) from the control group. (**Figure 2**)

Histological examination. Electron microscopic (EM) examination of the untreated rats showed regular arrangement of cardiac myocytes. The nuclei were oval, central in position with prominent nucleoli. Myofilaments were regularly arranged, dense, and continuously giving the striated appearance. Perinuclear mitochondria appeared organized and packed together. The mitochondria between myofilaments appeared stacked in rows in an orderly manner. They revealed transverse, parallel and regular cristae, and continuous mitochondrial membrane (**Figure 3** & **Figure 4**). The EM examination of the control group (following ISO stimulation) revealed marked distortion of the cardiac myocytes. Many nuclei appeared irregular and shrunken. The myofilaments were distorted, irregular, and discontinuous in many areas resulting in disruption of the regular striated appearance. The mitochondria were apparently decreased in number, both perinuclear and in-between myofilaments. The perinuclear mitochondria appeared disorganized and widely spaced. They appeared heterogenous with electron lucent areas, and showed very few cristae. The mitochondria between myofilaments appeared less in number, and were not uniformly stacked (**Figure 5** & **Figure 6**).

The BV-treated group. Examination of this group showed notable findings. The cardiac myocytes showed typical arrangement. The nuclei were regular in size and shape with prominent nucleoli. Myofilaments appeared regular, dense, and continuous giving the striated appearance. The perinuclear mitochondria appeared organized, and packed together with apparent increased in number as compared to the control group. They were also more electron dense in comparison to those in the control group. They revealed transverse, parallel, and regular cristae. The mitochondria between myofilaments appeared stacked in rows in orderly manner. The structure showed marked improvement as compared to the control group and similar to the untreated rats (**Figure 7** & **Figure 8**).

Discussion. This study of cardiac performance reflects the prevailing conditions at the time of the experiment. The present study showed insignificant ECG changes in the BV-treated group. In contrast, other research groups presented opposite findings. Nabil et al[11] demonstrated that at concentrations of 0.5-2 mg/ml, BV caused ECG changes, such as marked injury current, elevation or depression of the S-T segment, atrioventricular conduction disturbances, and sinus arrhythmias in isolated perfused toad hearts. In contrast, our data revealed that BV was not cardiotoxic

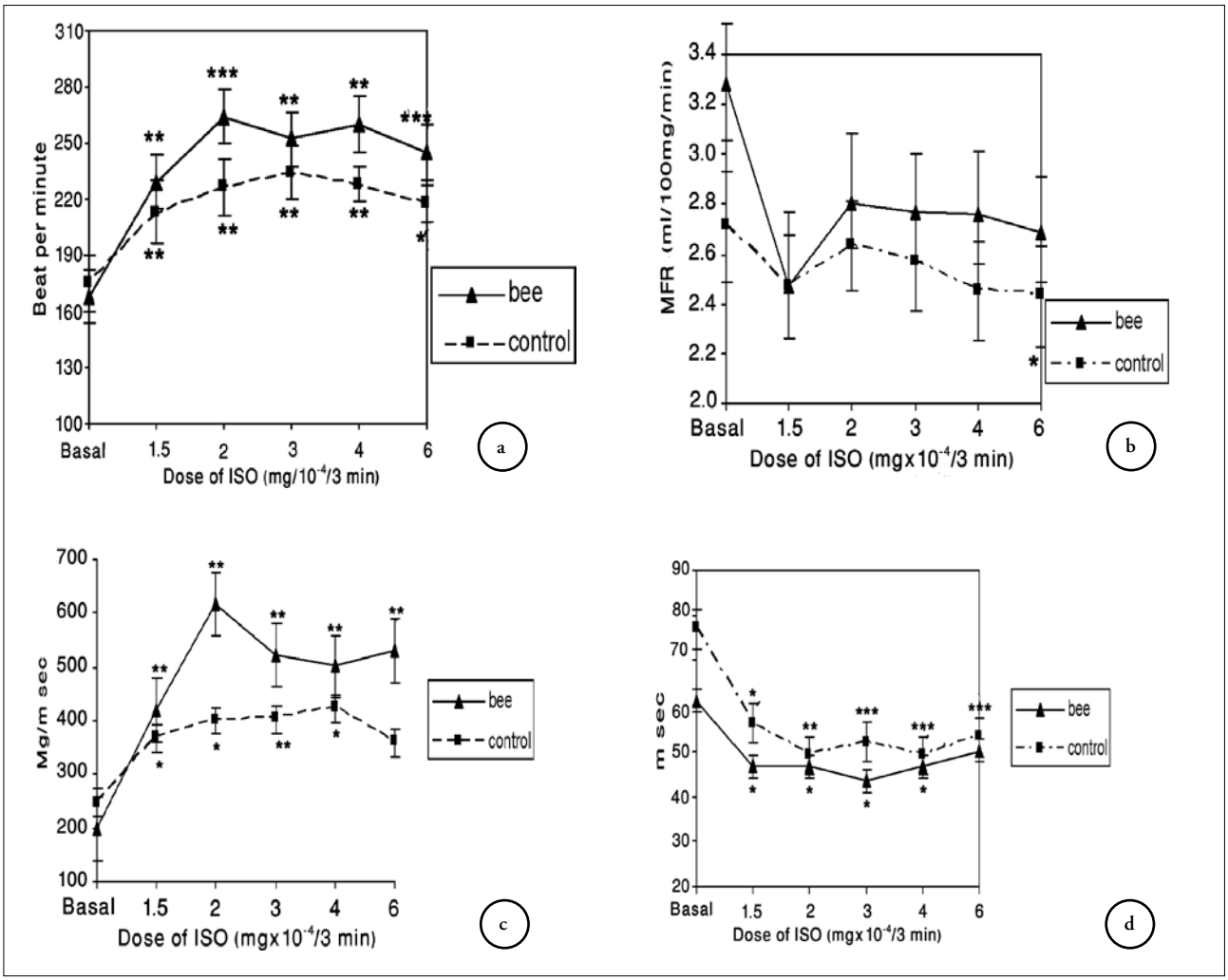

Figure 1 - Dose response curves to isoproterenol (ISO) infusion for different parameters of heart performance; **a**) heart beating rate, **b**) myocardial flow rate (MFR) per left ventricular weight; **c**) tension generation per unit time; and **d**) half relaxation time in the bee venom-treated and control groups. Data are means ± SEM. Significance of differences from the respective preinfusion value calculated by paired t-test, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

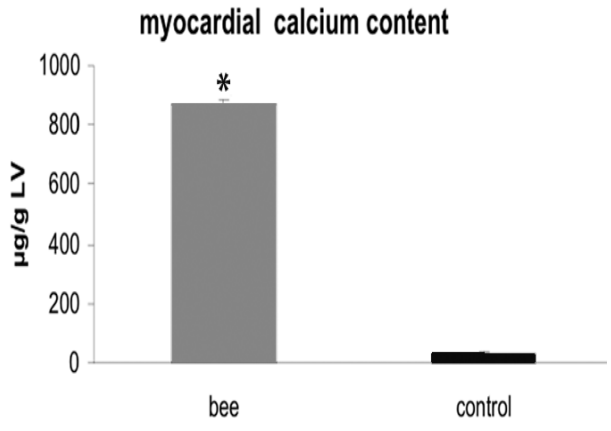

Figure 2 - Myocardial calcium content at the end of isoproterenol infusion (µg/g LV) in the bee venom-treated group and the control group. *Significance calculated by unpaired t-test at $p<0.05$ from the control group.

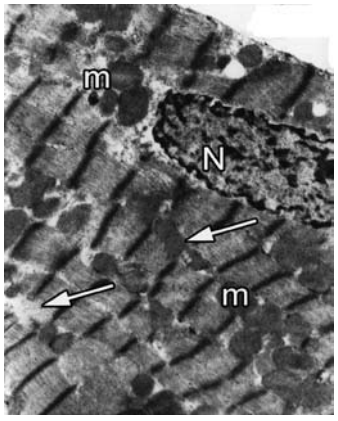

Figure 3 - An electron microscopic examination showing a cardiac myocyte in an untreated rat. The nucleus (N) appears regular and oval. The myofilaments (arrows) are continuous and regular. The mitochondria (m) appear in between the myofilaments and in the perinuclear space (untreated rat x7500).

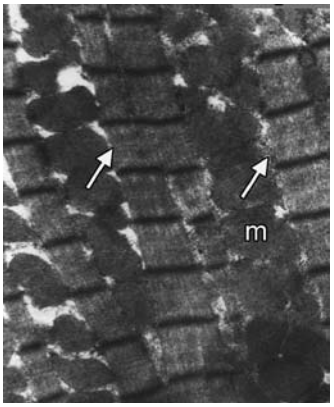

Figure 4 - An electron microscopic examination of the untreated rat showing regular, dense and continuous myofilaments (arrows). The mitochondria (m) are stacked regularly between the myofilaments (x10000).

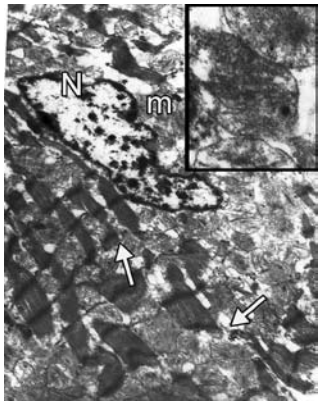

Figure 5 - An electron microscopic examination of the control group showing shrunken and irregular nucleus (N). Myofilaments appear, distorted, loose, and discontinuous (arrows). Perinuclear mitochondria (m) are irregular and widely spaced (x5000). Inset: Perinuclear mitochondria are heterogenous with electron lucent areas, and show very few cristae (x25000).

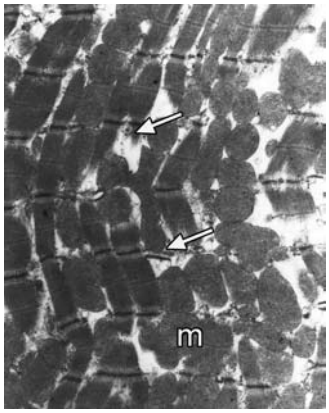

Figure 6 - An electron microscopic examination of the control group showing myofilaments (arrows) that appear disorganized and irregular. The mitochondria (m) are not uniformly stacked in between the myofilaments (x7500).

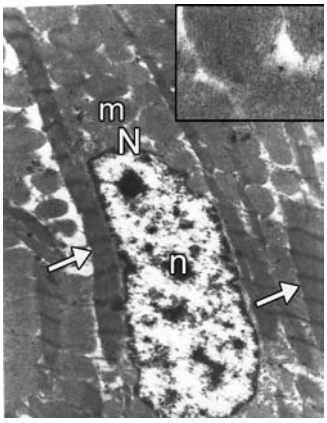

Figure 7 - An electron microscopic examination of the bee venom-treated group showing regular nucleus (N) with prominent nucleoli (n). Myofilaments (arrows) appear regular, dense, and continuous. Perinuclear mitochondria (m) are regular and closely packed (x5000). Inset: Perinuclear mitochondriaare more electron dense compared to the control group. They reveal transverse parallel cristae (x25000).

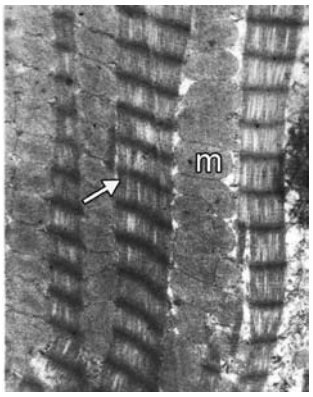

Figure 8 - An electron microscopic examination of the bee venom-treated group showing regular, dense, and continuous myofilaments (arrows). The mitochondria (m) are uniformly stacked in between the myofilaments (x7500).

at the applied dose. The controversy between different literature, which reported ECG changes induced by BV,[11] and our present study could be attributed to the large doses of BV used in these studies. The results of the present study showed overall non-significant changes in the baseline intrinsic activities of the isolated hearts after 4 days of BV supplementation. When these hearts were perfused with progressively increasing doses of ISO, they showed enhanced adrenergic responsiveness of the various cardiac parameters studied. Although these hearts showed a significant higher maximal dT/dt, they showed non-significant changes in their maximal HR, MFR, and ½RT compared to their matching control hearts. The selective action of BV on the inotropic properties

of these hearts excludes the possibility of recruitment of force-frequency relationship as an explanation for the enhanced inotropic reserve. The different cardiac adrenoceptor subtypes, properties, distribution and regulation,[12] could explain the differences between the inotropic effects of BV and the lusitropic and the chronotropic effects.

In an attempt to identify the underlying mechanism for the positive inotropic effect of BV, myocardial Ca^{2+} content was assessed. The Ca^{2+} was significantly increased in hearts isolated from the BV-treated group compared to their matching controls. The Ca^{2+} is necessary for myocardial function, including contraction and maintenance of cardiac output. The entry of extracellular Ca^{2+} depends mainly on Ca^{2+} channels, and the sarcolemmal Na/Ca exchanger system. Changes in calcium transport via Na/Ca exchanger system was reported to have negligible impact on the myocardium under physiological conditions.[13] Agonist-bound-adrenergic receptor (AR) increases cyclic adenosine monophosphate (cAMP) via adenylate cyclase activation (AC).[14] The cAMP via protein kinase A (PKA) phosphorylates the L-type calcium channel.[15] It has been reported that phospholipase A2 (PLA2) increased AC-activity in the rat caudate nucleus in a dose-dependent manner.[16] Similarly, it could be suggested that some of the BV components, that is PLA2, could have increased AC-activity in the rat cardiac muscle, thereby it increased Ca^{2+} entry through L-type calcium channel, however, further study is needed to confirm this assumption.

Calcium is also necessary for myocardial energetic and production of ATP by mitochondria.[17] The mitochondria rely on calcium to activate key dehydrogenases in the tricarboxylic acid cycle. This accelerates production of nicotinamide adenine dinucleotide, which provides a driving force for increase in proton motive force that maintains ATP production.[18] In cardiac myocytes, the mitochondria are responsible for meeting the cellular energy demands required to maintain excitation and contraction on a beat-to-beat basis.[19] It has been proposed that the mitochondria can rapidly tract changes in cytosolic calcium from beat-to-beat. Since the L-type Ca^{2+} is the initiator of contraction in cardiac muscle, Ca^{2+} entry through this channel has been proposed to represent a coordinated process, by which mitochondrial function is regulated on a beat-to-beat basis.[20] Therefore, in the current study, the observed increased myocardial calcium content could have enhanced the myocardial energetic, provided another mechanism for the positive inotropic effect of BV. Morphological degeneration of the cardiac cells induced by calcium overload has been demonstrated for some animal toxins. In this study, although the calcium content of myocardial cells was significantly increased in BV-treated rats, however, this increase did not induce morphological injury of myocytes. Beyond their role in generating ATP, the mitochondria has a high capacity to sequester calcium. The essential role in cellular response to Ca^{2+} overload is played by the mitochondria, behaving as temporary cellular safety devices in situations of Ca^{2+} overload, and mitochondria sequester calcium.[21,22] In this study, the pronounced increase in the number of mitochondria could have increased the capacity of the mitochondria to buffer the increased calcium load. Moreover, examination of the mitochondria at the end of ISO perfusion revealed preserved mitochondrial ultrastructural integrity, thereby providing an additional mechanism for the positive inotropic effect of BV.

In conclusion, the observed data demonstrated the cardiosafety of BV. Moreover, beneficial cardiac effects were demonstrated. The BV treatment was associated with an increase in the maximal tension generation upon progressive cardiac stress by ISO infusion. A possible cardiac mechanism of action of BV at mitochondrial level could support ATP synthesis by elevating the mitochondrial Ca^{2+} level. Concurrently, over-expression of the mitochondria in these hearts could provide local Ca^{2+} buffering effect, thereby protecting them against calcium overload induced damage. Although cardiac parameters provide evidence for safety and/or beneficial effect of BV, further and reliable human research is necessary before a recommendation can be made for the cardiosafety of clinical application of BV. The results of this study substantiate only the cardiosafety of short-term treatment with BV, further study should evaluate the cardiosafety of long- term treatment with BV.

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