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Effect of the bumblebee ' *Bombus morrisoni* ' venom on cardiac, skeletal and smooth-muscle activity

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ABSTRACT

The effect of bumblebee crude venom was studied on cardiac, skeletal and smooth-muscle activity. Three concentrations of the venom (0.5, 1 and 2 µg/ml) were applied on isolated perfused toad hearts. Significant bradycardia accompanied by positive dromotropism were obtained following the application of the three venom doses and sustained 30 min. A gradual and progressive increase in R-wave amplitude also resulted, reflecting positive inotropism of the venom. Application of verapamil, a calcium-channel blocking agent, apparently abolished the effect of the venom. Several cases of cardiac disorders were noticed after venom perfusion. Elevation and/or depression of the S-T segment was seen, indicating a direct toxic effect on the heart as ischaemia and infarct. Atropine and nicotine attenuated the toxic effect of the venom on the myocardium. Perfusion of toad gastrocnemius-muscle/sciatic-nerve preparation with 1µg/ml venom solution weakened the mechanical contraction of the muscle without any later recovery. Pretreatment with the nicotinic receptor antagonist sustained normal contraction of the skeletal muscle. Smooth-muscle activity was studied before and after treatment with bumblebee venom. Some isotonic changes in the contraction of rabbit duodenum were recorded after 15 min of venom application. Pretreatment with atropine could not abolish the effect of the venom on smooth muscle.

KEYWORDS: Bumble bee venom; ECG; muscles

INTRODUCTION

Bombus venoms are highly cross-reactive with honeybee venom since at least four allergens from honeybees can cross-react with similar allergens in *Bombus* (Hoffman 1982). The lethality of Hymenoptera venoms in mice varies greatly: the LD_{50} of *Bombus impatiens* is 7.2 µg/gm (Schmidt *et al.* 1980). Anaphylactic shock and strong cardiac

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stimulation is reported to be caused by stings of *Bombus terrestris* (Donnovan 1978). Hoffman (1982) reported induction of allergy by stings of bumblebees.

Early investigations on the venom components of bumblebees were noticed by Welsh & Batty (1963) who found very small amounts of serotonin in extracts of whole venom apparatus of unidentified *Bombus* species. About 30 µg of acetylcholine were collected from the tip of the sting of *Bombus terrestris* (Piek *et al.* 1983). The identification of the cholenergic factor with acetylcholine was confirmed using radioimmunoassay of acetylcholine. The venoms of *Bombus terrestris* and *B. lapidarius* also contain a component that causes slow contraction of the guinea pig ileum and rat diaphragm.

Five structurally related heptadecapeptides have been detected in the venom of the bumblebee *Megabombus pennsylvanicus*: bombolitin I, bombolitin II, bomolitin III, bombolitin IV and bombolitin V (Argiolas & Pisano 1985). Bombolitins lyse erythrocytes and liposomes, release histamine from rat peritoneal mast cells and stimulate phospholipase A_2 from different sources. They reported also that bombolitins represent a unique structural class of peptides, with the same biological properties as melittin.

Although investigations on bumblebee venom started a long time ago, certain questions concerning the manifestations of its toxicity as well as the role of central and peripheral nervous antagonists on the venom action are still uncertain. The present work was conducted to answer some of these questions.

MATERIALS AND METHODS

Venom collection

Venom was collected from the bumblebee *Bombus morrisoni.* Pure venom was obtained by the method of Schmidt (1986). Frozen bees were thawed, and the sting apparatus removed into a spot of distilled water. The venom reservoir was pinched off, removed from the rest of the sting apparatus, and rinsed with distilled water. Ten reservoirs were used in this investigation. The venom was squeezed out of the reservoirs, and the whole venom dehydrated over silica gel for 3 days. The resulting venom powder was dissolved in frog Ringer solution to a final concentration of 100 µg/ ml, and stored at - 20°C.

Animals

Adults male toads *'Bufo regularis'* 35-40 g body weight and male rabbits weighing about 0.5 kg were used in this investigation as an experimental model for the *in vitro* study.

In vitro experiments and doses

Cardiac muscle

Experiments on cardiac muscle were carried out on isolated toad-heart preparations. Three groups each of 10 animals were used. They were directly perfused with (0.5, 1 and 2 µg/ ml) venom solution. Electrocardiogram (ECG) data were recorded directly from the surface of the heart before venom application to serve as a self control. After venom perfusion, signals were recorded every five minutes for half an hour. ECG was recorded by a multi-pen rectilinear recorder (DBE, UK) with paper speeds of 2 and 10 mm/sec.

Skeletal muscle

Toad gastrocnemius-muscle/sciatic-nerve preparations (n =10) were used to study the effect of bumblebee venom on the mechanical contraction of skeletal muscle. Muscles were directly perfused with 1 µg/ ml of the venom solution. Activity of the skeletal muscle was recorded by an ink kymograph (model 10500, Bioscience, UK) with a paper speed of 1 mm/sec. The sciatic nerve was electrically stimulated with 1 V, 1.4 ms, 1 Hz square-pulse waves.

Smooth muscle

Rabbits were killed by a blow on the back of the neck: the abdomen was immediately opened, and the duodenum was excised and placed in a bowl of tyrode solution. A mono-organ bath with an inner vessel 40 mm was used where the gut preparation was mounted. The effect of the venom was studied by adding 1µg/ml venom solution to the bath. Electric activity of the muscle was recorded by means of a T3 auxotonic transducer, FC 100 direct-input coupler on a two-channel curvilinear oscillograph (model MD2, Bioscience, UK) with a chart speed of 0.25 mm/sec.

Antagonists

Atropine sulphate was purchased from an appropriate supplier (Memphis Co, Dorpharm and Chem, Ind. Cairo). A dose of 4 µg/ ml-Ringer was used on heart preparations, while 5×10^{-6} M in saline was added into smooth-muscle preparations. Nicotine with a large dose (1 %) was used on the myocardium (from Merck- Schuchardt, Egypt). Verapamil hydrochloride (40 mg: ADWIC Pharmaceutical Division, El Nasr Pharm. Chem. Co., Abu Zabal, Egypt), the calcium channel antagonist, was used on the heart with a dose of 5 µg/ ml. Flaxedil (manufactured by Alex. Pharm. Co., Egypt) was used on skeletal muscle at a dose of 3 µg/ml-Ringer solution.

Statistical analysis

Responses of heart rate (HR) and the other electrocardiographic parameters (P-R interval, R-wave amplitude) to venom treatments were expressed as means ± 1 standard error. Differences in the heart rate (HR) and other ECG parameters were analyzed using Student's paired t-test according to Snedecor & Cochran (1980). Differences between several doses were analysed by one-way analysis of variance (Anova), using $P < 0.05$ as a criterion of significance (Snedecor & Cochran 1980).

RESULTS

The effect of direct perfusion with the bumblebee (*Bombus morrisoni*) venom was studied on cardiac, skeletal and smooth muscles. Doses as low as 0.5-2 µg/ml venom solution induced changes in the physiological properties of isolated toad hearts within 20 mins after venom application, while higher doses immediately stopped the heart. Application of the venom with either of the three doses used (0.5, 1, and 2 µg/ml) resulted in severe bradycardia and an increase in the P-R interval, as well as increasing the R-wave amplitude. Several cases of cardiac disorders were noticed after venom application. Table 1 shows the effect of direct perfusion (with doses of 0.5, 1, and 2 µg/ml) of bumblebee venom on the heart rate of the toad heart. The venom induced significant bradycardia, which began from the onset of venom application and lasted 30

min at all three dose levels, with no significant dose-dependent relationship (Anova, P>0.05).

Table 1: Effect of direct perfusion with bumblebee *(Bombus morrisoni)* venom on heart rate of isolated toad hearts. Values represent means ± se (n=10/group).
* implies that a value is significantly different from the value for time = 0
paired t-test, p<0.05). Heart rates taken at the same time do not differ among doses (Anova, p > 0.05).

Time (min)	Heart rate (beats/min)		
	0.5 µg/ml	1 µg/ml	2 µg/ml
0	62.9 ± 2.6	59.6 ± 2.0	55.5 ± 2.7
5	50.3* ± 2.4	46.9* ± 1.4	46.0* ± 2.4
10	42.1* ± 4.0	42.2* ± 2.7	41.6* ± 2.1
20	43.7* ± 4.3	31.6* ± 4.0	34.3* ± 3.4
30	36.3* ± 4.2	31.0* ± 6.4	30.9* ± 3.2

Table 2 shows the effect of venom application on conduction time (P-R interval). A significant decrease in the conduction velocity at all time intervals followed venom application at all three doses (Student's t-test, P<0.05) with non-significant variance between the doses. Table 3 shows the effect of venom on myocardiac contractility (R- wave amplitude): contractility increased significantly with a maximum value after 5 min following perfusion at each of the three dose levels, with no dose-dependent relationship (Anova, P>0.05). The effects of the venom are illustrated in electrocardiograms (Plate 1) taken from different isolated hearts treated with the three doses of bumblebee venom. Plate 2 demonstrates several cases of cardiac disorders noticed after venom perfusion on isolated hearts. These cases are representative of different types of arrhythmias, such as abnormal sinus rhythm, different degrees of A-V block and S-T segment elevation and/or depression (such as ischaemia and infarct). The incidence of cardiac disorders are tabulated in Table 4.

Table 2: Effect of direct perfusion with bumblebee *(Bombus morrisoni)* venom on the P-R interval of isolated toad hearts. Values represent means ± se (n=10/group).
* implies that a value is significantly different from the value for time = 0
paired t-test, p<0.05). Heart rates taken at the same time do not differ among doses (Anova, p > 0.05).

Time (min)	P-R interval (ms)		
	0. 5 µg/ml	1 µg/ml	2 µg/ml
0	350 ± 34.2	370 ± 26.0	390 ± 23.3
5	500* ± 33.3	540* ± 22.1	510* ± 27.7
10	570* ± 36.7	590* ± 23.3	570* ± 36.7
20	657.1* ± 48.1	650* ± 37.8	640* ± 42.7
30	700* ± 68.3	680* ± 58.3	675* ± 41.2

Table 3: Effect of direct perfusion with bumblebee *(Bombus morrisoni)* venom on the R-wave amplitude of isolated toad hearts. Values represent means ± se (n=10/group).
* implies that a value is significantly different from the value for time = 0
paired t-test, $p<0.05$). Heart rates taken at the same time do not differ among doses (Anova, $p > 0.05$).

Time (min)	R-wave amplitude (mv)		
	0.5 µg/ml	1 µg/ml	2 µg/ml
0	1.32 ± 0.15	1.10 ± 0.14	1.34 ± 0.08
5	1.68* ± 0.14	1.48* ± 0.16	1.66* ± 0.05
10	1.58* ± 0.13	1.40* ± 0.15	1.64* ± 0.09
20	1.54* ± 0.17	1.28* ± 0.14	1.38* ± 0.10
30	1.33* ± 0.29	1.08* ± 0.21	1.28* ± 0.17

Table 4: Incidences of ECG abnormalities as a result of bumblebee *(Bombus morrisoni)* venom administration (n=10/group).

Cardiac Disorder	Number of incidences		
	0.5 µg/ml	1 µg/ml	2 µg/ml
(A) Abnormal sinus rhythm:			
- Bradycardia:	10	10	10
- Sinus arrhythmias:	1	2	3
(B) Ectopic beats:			
- Atrial escape:	-	2	-
- Junctional escape:	5	4	6
- Premature atrial contraction:	1	-	-
(C) Atrioventricular block:			
- First degree:	10	10	10
- Second degree:	3	4	3
- Complete block:	3	2	-

The mechanism of action of *Bombus morrisoni* venom on toad heart was studied by blocking the cholinergic receptors or ganglia with either atropine or nicotine, as well as the calcium channels by verapamil. Plate 3 shows the effect of adding atropine (1), nicotine (2), or verapamil (3) onto toad hearts perfused with 1 µg/ml of venom solution. Cases of second and complete heart block as a result of the venom application started beating after adding atropine or nicotine (part 1& 2). In the same plate, the positive inotropic effect of the bumblebee venom was abolished by verapamil application (part 3).

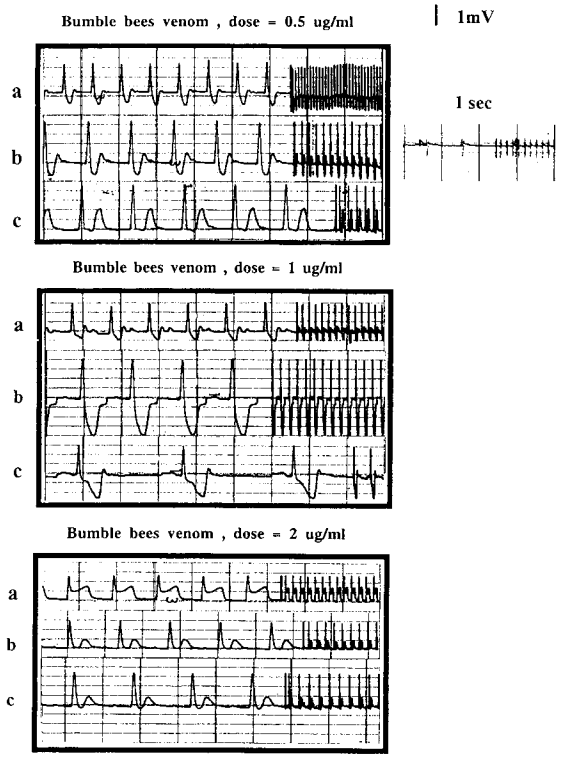

Plate 1: Electrocardiograms showing the effect of different doses of *Bombus morrisoni* venom on isolated perfused toad hearts. **a:** Before treatment **b:** 10 min after treatment. **c:** 30 min after treatment.

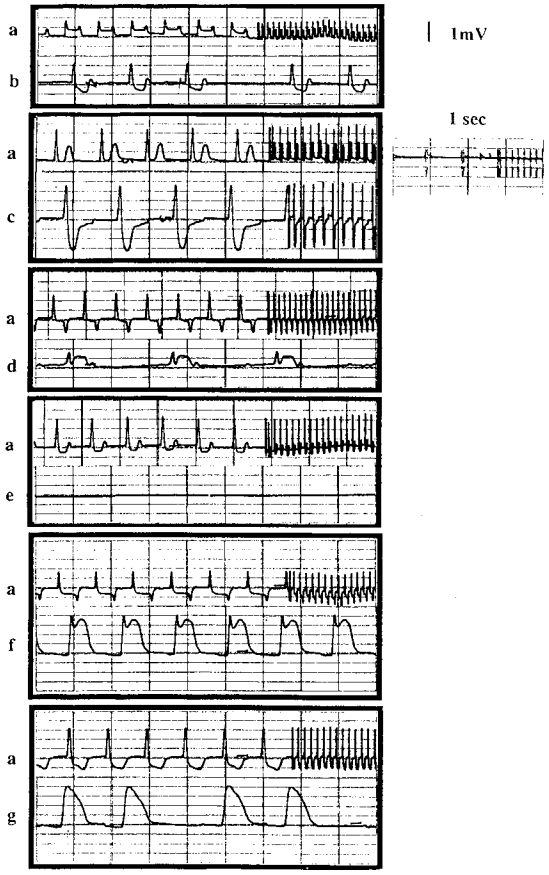

Plate 2: Examples of cardiac disorders in isolated toad hearts as a result of *Bombus morrisoni* venom application. **a:** Normal trace. **b:** Sinus arrhythmias. **c:** First degree block. **d:** Second degree block **e:** Complete heart block **f:** Ischaemia **g:** Infarct.

The activity of toad skeletal muscle (gastrocnemius muscle) was studied before and after bumblebee venom application (1 µg/ml). Plate 4 (I) shows the decrease in muscle contraction following venom perfusion. Blocking the nicotinic receptors with 3 µg/ml flaxedil before venom application almost sustained normal contraction of the muscle.

The activity of the smooth muscle (duodenum of rabbits) was also studied before and after bumblebee venom application (1 µg/ml). Plate 4 (II) shows some isotonic changes in the muscle contraction following venom perfusion. Blocking the muscarinic receptors with atropine before venom application did not noticeably abolish the effect of the venom.

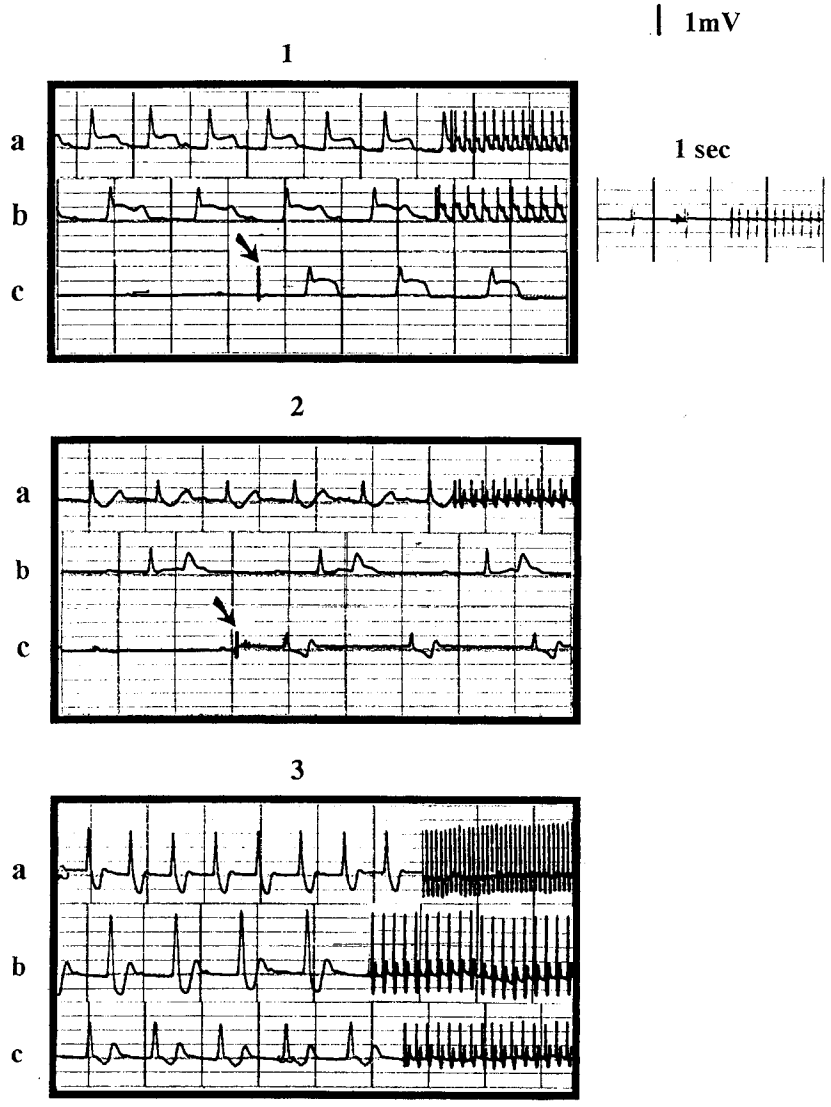

Plate 3: Effect of adding antagonists into isolated toad hearts perfused with 1µg/ml of *Bombus morrisoni* venom solution.

1- Atropine. **2-Nicotine.** **3- Verapamil.**

a- Before venom application.

b- 10 min after venom application.

c- After adding the antagonist.

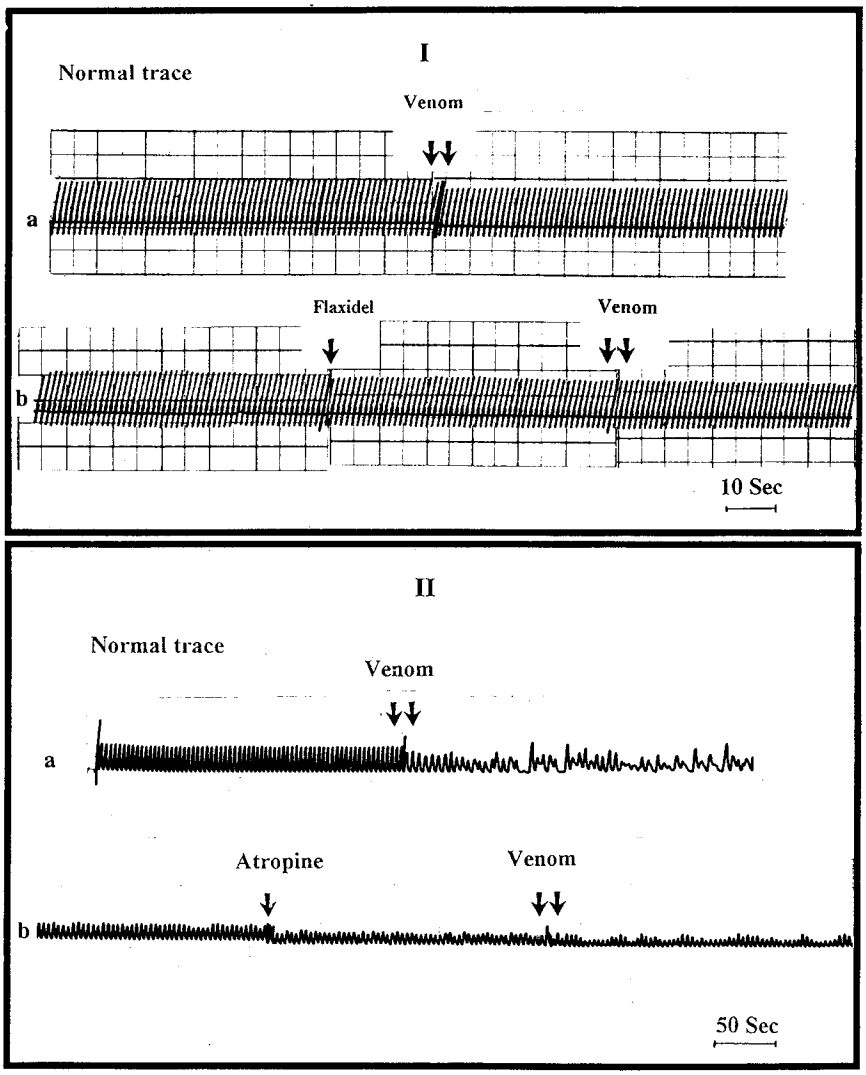

Plate 4: Effect of direct perfusion with 1µg/ml of bumblebee (*Bombus morrisoni*) venom solution on the:
I- Mechanical activity of the toad gastrocnemius muscle.
II- Electric activity of the smooth muscle of rabbit duodenum.
a: Effect of the venom.
b: pretreatment with antagonist.

DISCUSSION

Hymenopterans are biologically a very interesting group bceause of their omnipresence in practically every habitat. Medically, they comprise the most significant venomous animals of the world since their stings are the cause of the majority of deaths (Meier 1995). There are nine currently recognised families in the superfamily Apoidea, but only the bumblebees (subfamily Bombinae) and honeybees (subfamily Apinae) from the family Apidae are of medical importance (Meier 1995). Our previous investigation (Nabil *et al.* 1998) studied the mechanism of action of honeybee (*Apis mellifera* L.) on different types of muscles. The present investigation introduced some important results about bumblebee (*Bombus morrisoni*) venom. Striking effects on the ECG taken from isolated toad hearts were noticed after direct perfusion even at the low concentrations used (0.5- 2µg/ml). Negative chronotropism and dromotropism are the main effects

induced by bumblebee venom on the myocardium, even at low doses since no dose-dependent effects were obtained. Moreover, several cases of cardiac disorders were demonstrated during the experiments, concluded to be as a result of the progressive negative effect of the venom, such as the atrioventricular block after cases of bradycardia, as well as ischaemia and infarct after S-T elevation or depression.

A positive inotropism of bumblebee venom was obtained 10 min after venom application for all of the doses used. This was manifested by an increase in R-wave amplitude in the ECG record of the isolated toad hearts, indicating an increase in their contractile force (stimulatory effect). Donnovan (1978) reported strong cardiac stimulation by bumblebee stings (*Bombus terrestris*), which agrees with the present data.

A decrease in contraction of skeletal muscle perfused with bumblebee venom solution was noticed, which indicates the relaxant effect of this venom. In addition, some isotonic changes induced in the activity of the treated smooth muscle following the venom perfusion reflect its ability to impair the tone of the muscle.

The skeletal muscle nicotinic receptor antagonists, parasympathetic antagonists for both cholinergic and muscarinic receptors, as well as the cardiac calcium channel antagonists used in this investigation has established the mechanism of action of the bumble bee venom on the skeletal, smooth and cardiac muscle activity. Hence, the depressor effect of the venom on the skeletal and cardiac muscle activity was abolished by the application of flaxidel and atropine, respectively. In the meantime, atropine could not abolish the isotonic changes induced by bumblebee venom on smooth muscle, which consequently reflects direct action of the venom. This agrees with Piek *et al.* (1983) who noticed that the effect of bumblebee (*Bombus terrestris*) venom on guinea-pig ileum was not antagonized by atropine. In the same manner, verapamil abolished the stimulatory effect of the venom on the voltage of ventricle, indicating that the positive inotopism is due to Ca^{2+} influx. Propranolol could not abolish the increase of the R-wave amplitude obtained in our results, which confirms the direct effect of the venom on the heart. Argiolas & Pisano (1985) have found five structurally related heptadecapeptides in bumblebee (*Megabombus pennsylvanicus*) venom (bombolitins I-V). They attributed the release of histamine from rat peritoneal mast cells and stimulation of phospholipase A_2 from different sources to bombolitins. Moreover, they concluded that bombolitin V is as potent as melittin from honeybee venom. The above conclusion about the amphiphilic nature of both honeybee and bumblebee venoms is the reason for the matching of the present data on bumblebee venom with previous data for honeybees (Nabil *et al.* 1998) since both of the two venoms tend to have the same effect on cardiac and skeletal muscle. However, the effectiveness of the two venoms is different, and their action on smooth muscle is not identical.

In conclusion, *Bombus morrisoni* venom affects smooth muscle directly and has a dual effect on the myocardium through its direct and indirect action. The venom affects the neuromuscular-junction of skeletal muscle, probably through the nicotinic receptors.

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الملخص العربى

تأثير سم النحل الطنان "*بومبيس موريسونى*" على نشاط العضلات القلبية والهيكلية والملساء

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تم دراسة تأثير السم الخام للنحل الطنان "*بومبيس موريسونى*" على نشاط العضلات القلبية والهيكلية والملساء. وقد تم إستخدام ثلاث جرعات من السم (٠,٥ ، ١، ٢ميكروجرام/مل) على القلوب المفصولة للضفدع. كان للجرعات الثلاث للسم تأثير إيجابى فى هبوط القلب المصاحب بالزيادة الإيجابية فى المسافة التوصيلية لنبضات القلب والذى يعنى نقص سرعة التوصيل. وقد ظل التأثير مستمراً لمدة نصف ساعة بعد وضع السم. أيضا نتج عن تأثير السم زيادة إيجابية ومطردة فى الجهد القلبى وقد تمثل ذلك فى إرتفاع موجة الـ R فى رسم القلب الكهربى والذى يعكس ظاهرة السم الإيجابية للجهد البطينى. وقد أختفى تأثير السم على الجهد البطينى بإستخدام الفيرابراميل المضاد لممرات الكالسيوم.

كان للسم تأثير ملحوظ فى ظهور عدة حالات من الخلل الوظيفى للقلب. وكان إرتفاع أو إنخفاض موجة S-T فى رسم القلب دليل على التأثير المباشر للسم على القلب. وقد إستطاع الأتروبين والنيكوتين خفض وإزالة تأثير السم على العضلات القلبية. وكان لغمر العضلة الهيكلية الساقبطنية للضفدع بالسم إنخفاض ملحوظ فى إنقباضها من غير إرجاع. بينما المعالجة السابقة للعضلة بمضادات المستقبلات النيكوتينية قد أظهر إنقباضا طبيعيا لها.

تم أيضا دراسة تأثير سم نحل الطنان على نشاط العضلات الملساء. وقد أظهرت المعالجة بالسم تغيير وإضطراب فى النشاط الكهربى للفائفى الأرنب بعد ربع ساعة من وضع السم ولم يزيل الأتروبين تأثير السم على تلك العضلات الملساء.