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Activity and pharmacology of the venom of *Proxylocopa rufa*, a primitive anthophorid bee

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ABSTRACT

The venom of the large bee, *Proxylocopa rufa,* induces symptoms in envenomed mice unlike those observed for any other insect venom. These include immediate exophthalmia, rapid cyanosis, hyper "fidgeting", persistent clonic convulsions, and death subsequent to respiratory paralysis. The heart muscle appeared paralysed by the venom, but the lungs remained primarily unaffected. The venom lethality of 11.3 mg/kg falls into the moderately toxic range for animal venoms. The venom effects on the cardiovascular and central nervous systems are indications that this unusual species of bee not only has an interesting taxonomic position among the bees, but also it might have evolved specialised and unique venom components.

KEYWORDS: *Proxylocopa rufa*, Anthophoridae, Hymenoptera venom, lethality, toxin, cardioactivity

INTRODUCTION

Proxylocopa is a small group of Old World bees found in the arid southwestern Palaearctic, with several species in the Middle East. It belongs to the tribe Xylocopini which it shares with the enormous group *Xylocopa*. Although *Proxylocopa* is often considered to be an ancestral lineage relative to *Xylocopa* (Hurd & Moure 1963), its exact relationship is unclear and it might actually be a subgenus of *Xylocopa* (Minckley 1998). Unlike *Xylocopa sensu stricto*, which nests in wood, hence their common name of carpenter bees, *Proxylocopa* nests in soil or hard earth. Little detailed knowledge of these often nocturnal or crepuscular and uncommonly encountered bees is known and nothing is known of their venoms.

Proxylocopa are rather large bees, measuring 18-20 mm in length, and like most

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bees they feed themselves and their larvae exclusively on nectar and pollen. For this reason, their venoms are not used for killing or paralysing prey; rather it is used solely for defence against predators, especially large vertebrate predators. The purpose of this investigation is to determine the potential defensive value of the venom of one species, *P. rufa* (Friese), by analysing its lethality and effects on potential mammalian predators, using the mouse as the model system.

MATERIALS AND METHODS

Foraging bees were collected by insect net from 16-18 May, 1996 in Wadi Arbaein near the Environmental Research Center (ERC), St. Katharine, Sinai, Egypt. Both females and males were captured from 05:30 - 06:30 and 19:00 - 20:00 hours while actively foraging on *Capparis spinosa* L. (Capparidaceae). Captured bees were placed in tubes, transported back to the Station, frozen, and dissected within two days to obtain venom.

Venom was collected by the method of Schmidt (1986). In brief, the sting apparatus with attached venom reservoir is removed and placed in a droplet of distilled water. Forceps are used to tease the venom reservoir free of surrounding tissues, the duct of the reservoir leading to the sting base is pinched closed with forceps and the reservoir is removed to another droplet of distilled water. While still holding the duct tightly, the reservoir is then rinsed in the second droplet to remove any contaminating haemolymph or cellular debris. The reservoir is finally transferred to a third water droplet and released. After the desired number of reservoirs are collected into the final droplet, all are gently torn with forceps, the venom allowed to drain and the empty reservoirs removed. The venom is then placed in polyethylene micro tubes (BioRad, Richmond, Calif.) and dried over silica gel. Drying typically requires from 12-24 hours at ambient temperature.

Lethality and gross morphological studies were conduced with the SWV/W1 strain of inbred white swiss mice. Doses of 4, 8, and 16 mg venom/kg body weight were administered intravenously in the lateral tail veins of six mixed-sex mice per dose. Venom was dissolved in 0.15 M saline and the injected volumes ranged from 1 to 4 µl venom solution/g body weight. Reactions and times of death of mice were recorded, and mice were dissected within two minutes of death for gross pathological observations. LD_{50} values at 24 hours were calculated by the method of Reed & Muench (1938).

RESULTS

Mice injected with venom of *Proxylocopa rufa* were almost instantly affected. The immediate reaction was profound exophthalmia which endured for about 5 minutes. This stage was followed by extreme cyanosis. Despite the cyanosis the animals were fidgety and constantly moved about in the cages in a non-directed manner. After this, individuals would frequently exhibit powerful clonic convulsions, typified by the animal rolling onto its back or side and rigidly extending all four limbs followed by relaxation and repeat. These convulsions continued sporadically and persistently for many minutes. At dosages of 16 mg/kg animals survived from 7 to 15 minutes; at 8 mg/kg all animals survived. These latter

animals all looked impaired for 1-2 hours, but by 8 hours all appeared perfectly normal and suffered no subsequent effects. Those animals that received 4 mg/kg (about 35% of a median lethal dose) were severely affected for at least an hour with cyanosis and some clonic convulsions before uneventful recovery.

Death was preceded by respiratory paralysis. After breathing stopped, and for about 30 seconds thereafter, affected animals often exhibited a series of strong contractions of the peritoneal muscles in what appeared to be an attempt to move air in and out of the lungs. When these contractions ceased, the heart and lungs were revealed and observed. In all cases, the lungs were white-pink and not congested. The heart ventricles were still except for a "shimmering" effect of random independent muscle cell contractions. The auricle continued contractions which lasted 25 and 33 minutes in the two animals recorded. The LD_{50} of the venom was 11.3 mg/kg with a 95% confidence range of 7.2-17.8 mg/kg.

DISCUSSION

The lethality of *Proxylocopa rufa* venom falls within the middle range of those reported for insect venoms. Insect venoms range in recorded lethality to mice from less than 0.15 to greater than 100 mg/kg, with bees falling in the range of 2.8 to 76 mg/kg (Schmidt 1986 & 1990, unpublished data). The most toxic of the bee venoms are those of the social bees, with *Apis* the highest at 2.8 mg/kg (Schmidt 1995) followed by *Bombus*. The bees in the Xylocopini including *Xylocopa* and *Proxylocopa* are large bees that exhibit behaviours ranging from solitary to the earliest stages of sociality. The lethalities of four species of *Xylocopa* range from 21 to 33 mg/kg (Schmidt, 1990 unpublished). The lethality of *Proxylocopa* venom is greater than that of any recorded species of *Xylocopa*, perhaps a surprising finding since *Proxylocopa* traditionally has been considered more primitive than *Xylocopa,* and because the lethality of insect venoms tends to increase with increasingly derived social behaviour (Schmidt 1990).

The lethality of *Proxylocopa* venom is, in itself, not particularly noteworthy. However, the surprising features of the venom are the symptoms induced in envenomed animals. Exophthalmia is induced by some insect venoms, particularly by the venoms of *Pogonomyrmex* ants, but the degree and immediacy of the effect is striking for *Proxylocopa* venom. Cyanosis is rather frequently observed as a symptom of envenomed animals, but again the rapidity of the onset of cyanosis and its severity in mice envenomed with *Proxylocopa* venom is remarkable. Although apneic convulsions are commonly observed in mice challenged with insect venoms, clonic convulsions are much less frequently noted. The clonic convulsions of mice subjected to *Proxylocopa* venom are further differentiated from those seen with other insect venoms by their persistence and often failure to cause death. When observed in animals envenomed with other insect venoms, death typically rapidly follows the convulsions. The clonic convulsions induced by the venom of *Proxylocopa* venom are much stronger, shorter in duration, and appear distinctly different from the long-term, persistent, uncontrolled tremors observed in mice challenged with honey bee (*Apis mellifera*) venom or its component apamin (Schmidt 1995). Finally, paralysis of the diaphragm followed by apparent attempts to respire via abdominal

contractions has never been reported before with insect venoms.

This investigation cannot provide definitive answers to questions about how *Proxylocopa* venom kills the animal. It does provide some tantalising information for speculation. Because the lungs were not congested, but appeared relatively normal, and apneic gasping was not observed, the venom appears not to target the lungs directly, or their ability to transfer oxygen. The fact that extreme, rapid cyanosis was observed suggests that oxygen transfer was, nevertheless, severely impaired. Exophthalmia in mice can be caused by rapid and severe increases in blood pressure which tend to fill and overexpand the orbital sinuses forcing the eyes outward. These two observations suggest that the venom targets the heart or vascular system and may cause an initial hypertension, followed by failure. The failure could cause the lack of oxygenation to occur. When mice that just died after venom challenges from other insect species are examined, their hearts are frequently still beating, or fibrillating. The hearts of mice that had just died of *Proxylocopa* venom were without contractions or fibrillations and exhibited only minor fasciculations, suggesting that their hearts might have been paralysed directly by the venom. A supporting observation is that the heart auricle continued beating for about half an hour after the animals had died. Thus the auricle tissue was not strongly affected by the venom.

Proxylocopa venom appears to contain factors that target the central nervous system as well as the cardiac muscle. Clonic convulsions and apparent paralysis of the diaphragm could be indicative of action on the central nervous system. If a central effect is present, it appears not to impede the function of noncardiac muscles.

Overall, we know nothing of the biochemistry of *Proxylocopa* venom, but its pharmacological activities indicate that the venom might contain factors that act centrally on the nervous system and directly on the cardiovascular system. These reactions are different from those induced by any other known insect venom and suggest that new, different, and potentially interesting components are present in *Proxylocopa* venoms.

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الملخص العربى

النشاط والتأثير الفارماكولوجى لسم نحل *"بروزيلوكوبا روفا"* (نوع بدائى من النحل البرى) على فئران التجارب

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تم دراسة تأثير سم نوع من النحل البرى كبير الحجم *"بروزيليكوبا روفا"* على فئران التجارب ولقد وضح أن الأعراض التى يسببها السم عند الحقن فى الفئران مختلفة بشكل كبير عن تأثير سموم الحشرات الأخرى حيث صاحب حقن الفئران بالسم ظهور الأعراض التالية: ضيق سريع فى التنفس، زرقة فى البشرة، قلق شديد، وتشنج إرتجافى دائم، وفى النهاية حدوث وفاة نتيجة شلل بالجهاز التنفسى بالرغم من عدم تأثر الرئات، أيضا حدث شلل فى عضلة القلب.

أوضحت الدراسة أن سمية هذا النوع تصل إلى ١١.٣ مجم/كجم والتى تعتبر درجة متوسطة السمية مقارنة بسموم الحشرات الآخرى. وضح أيضا أن السم ذو تأثير على الجهاز الدورى والجهاز العصبى المركزى مما يؤكد أن هذا النوع بالإضافة إلى أنه يحتل مكانة تصنيفية متميزة بين أنواع النحل الآخرى فإن سمه ربما يحتوى على مركبات متميزة وفريدة.