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【Scientific note】

亞非馬蜂 (Polestes rothneyi grahami)及陸馬蜂 (P. olivaceus) 蜂毒藥理活性之初步報告【科學短訊】

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Abstract

摘要

本研究分析兩種重要的中國馬蜂：亞非馬蜂 (Polistes rothneyi grahami)及陸馬蜂 (P. olivaceus) 蜂毒的藥理活性、純度及對人類的潛在價值。P. rothneyi grahami 及P. olivaceus 之蜂毒注入鼠體後活性頗高；致死中量 (LD50) 分別為14.5mg / kg 及11.2mg / kg。以等電點電泳法 (isoelectric focusing)分析蜂毒可見許多蛋白質帶，其中多數為鹼性。蜂毒的差異顯示兩種馬蜂蜂毒的組成和潛在活性均不相同。P. olivaceus 的蜂毒對哺乳動物白血球細胞的破壞力不強，而且其磷脂酵素也低，由此可見兩種蜂毒對哺乳動物組織的損害程度小，值得詳加分析，並進而測試其在醫藥學上之利用價值。

Key words:
關鍵詞: 亞非馬蜂、陸馬蜂、蜂毒、溶血、磷脂酵素。

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Pharmacological Activities of *Polistes rothneyi grahami* and *P. olivaceus* (Hymenoptera: Vespoidea) Venoms, a Preliminary Report

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ABSTRACT

The venoms of two important species of Chinese paper wasps, *Polistes rothneyi grahami* and *P. olivaceus* were analyzed for pharmacological activity. Both venoms were moderately active when injected into mice, with repective LD_{50} values of 14.5 and 11.2 mg/kg. When analyzed on isoelectric focusing (IEF) numerous protein bands were observed with many being highly basic. The venoms showed differences indicating that the two species have different compositions and activities. The venom of *P. olivaceus* possesses weak damaging potential toward blood cells and low levels of the enzyme phospholipase. These low values suggest that minimal tissue damage will result from injection of the venoms into mammals and that the two venoms merit detailed analysis and testing for potentially beneficial medical or pharmacological activities.

Key words: *Polistes rothneyi grahami*, *Polistes olivaceus*, venom, phospholipase, hemolysis

亞非馬蜂(*Polistes rothneyi grahami*)及陸馬蜂(*P. olivaceus*)蜂毒藥理活性之初步報告

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摘 要

本研究分析兩種重要的中國馬蜂：亞非馬蜂(*Polistes rothneyi grahami*)及陸馬蜂(*P. olivaceus*)蜂毒的藥理活性、純度及對人類的潛在價值。*P. rothneyi grahami* 及 *P. olivaceus* 之蜂毒注入鼠體後活性頗高；致死中量(LD_{50})分別為 14.5 mg / kg 及 11.2 mg / kg。以等電點電泳法(isoelectric focusing)分析蜂毒可見許多蛋白質帶，其中多數為鹼性。蜂毒的差異顯示兩種馬蜂蜂毒的組成和潛在活性均不相同。*P. olivaceus* 的蜂毒對哺乳動物白血球細胞的破壞力不強，而且其磷脂酵素也低，由此可見兩種蜂毒對哺乳動物組織的損害程度小，值得詳加分析，並進而測試其在醫藥學上之利用價值。
關鍵詞：亞非馬蜂、陸馬蜂、蜂毒、溶血、磷脂酵素。

Wasp, bee, and ant venoms have been used widely as traditional medicine for treatment of a variety of diseases and ailments, ranging from allergy to rheumatoid arthritis (Schmidt, 1986). Most emphasis has been placed on honey bee venom, particularly in reference to its effects on rheumatoid arthritis (Schmidt, 1992), with less emphasis on the venoms of ants (Schultz and Arnold, 1977). Although wasp venoms have been reported to be used by people living in the Brazilian Amazon region for treatment arthritis (Bequaert, 1926), no scientific information is available on the medical potential of wasp venom. Wasp venoms are known to contain pharmacologically active compounds (Nakajima, 1986). Most of these compounds are poorly characterized and their activities explored relatively little compared to those of snake and honey bee venoms.

The purpose of this research collaboration is to analyze the venoms of two common and potentially useful wasps, *Polistes rothneyi grahami* and *P. olivaceus*. Reported here are the preliminary finding of this research.

Wasps were collected and their venom obtained by the second author. Dried venom was shipped to Tucson, AZ where analyses were performed. The venom was then dissolved in small amounts of distilled water and filtered through 0.45 micron filters to sterilize it and remove any particulate. This venom was tan in color and was freeze dried and stored at -20℃ until further analysis.

Lethality tests were conducted with four groups of six mice per venom. The mice were given graded doses of venom in 0.9 % saline by intravenous injections into the tail vein. Median lethal dose (LD_{50}) analyses and calculations were

performed as described in Schmidt *et al.* (1986).

Hemolytic assays were performed by radial diffusion assay. Agarose gels (0.6%) containing 0.25% thrice washed human erythrocytes in 10 mM Tris-HCl pH 7.7 buffered saline were poured to a depth of 2 mm into plastic petri dishes. After cooling, 8μl wells were punched and graded doses of venom and melittin standard were added to separate wells and incubated 4 h at 24℃. Activity was determined by comparison of the diameters of the lysed circles to those of the standard. The standard used was a reference honey bee venom. Hemolytic activity is proportional to the log of the diameter of the lysed circles.

Phospholipase activities were determined by radial diffusion assay as described in Schmidt *et al.* (1986). Briefly, 0.6% agarose was dissolved at 90 ℃ in 50 mM Tris-HCl pH 8 buffered saline containing 10 mM $CaCl_2$. When the solution cooled to 50℃, 2% egg yolk was added to provide the substrate source of phospholipid and the solution was immediately poured into diffusion plates. Wells were punched in the plates and graded doses of venom or standard added and allowed to diffuse at 22-23℃ for 24 h. Activity is recorded as the diameter of the generated clear yellow circles in the cloudy yellow agarose matrix. Activity was quantified relative to a reference honey bee venom of known activity. Phospholipase activity is proportional to the log of the diameter of the cleared circles. Activity is expressed as equivalents of honey bee phospholipase.

Isoelectric focusing was performed on a cooled flatbed gel electrophoresis apparatus. Polyacrylamide gels containing 5.2% pH 3.5-10 ampholyte in 3 M urea were focused for four hours at voltages that increased from 200 v to 400 v. The protein bands were fixed in 12% trichloroacetic acid and stained with silver stain.

Both *Polistes r. grahami* and *P. olivaceus* venoms are highly active toward mice. Their respective LD_{50} values plus 95% confidence intervals are 14.5±7 29 mg/kg and 11.1±4.5-27 mg/kg. The venom of *P. olivaceus* is quite low in hemolytic activity relative to honey bee venom. Compared to the standard honey bee venom which contains on average 31.6% melittin, *P. olivaceus* venom contains only 0.9% melittin equivalent. The phospholipase activity of the venom is also low compared to honey bee venom. The standard honey bee venom contains 10.6% phospholipase, in comparison to only 2.2% bee venom phospholipase equivalent in *P. olivaceus*.

Figure 1 shows the banding pattern of the venoms of both species of *Polistes* on a pH 3.5-10 electropherogram. The complex protein nature of *P. r. grahami* (lane 7) and *P. olivaceus* (lane 8) can be clearly observed. Difference between these two venoms can be observed as well as major differences from honey bee venom. In particular, neither wasp venom contains melittin, the heavy and most basic visible band at the top of the lanes containing honey bee venom. *Polistes olivaceus* venom also contains a prominent band near pH 7.5 which is not visible in the various honey bee venoms or fractions.

The lethal activity of only one Old World species of *Polistes* has been reported previously, that being *P. tepidus*, a large species collected in Papua New Guinea. The LD_{50} of that venom is 7.7 mg/kg (Schmidt, 1990), about 50-70 percent as large as the two species in this study. Several New World species have been investigated and lethalities range from about 1.3 to 11.3 mg/kg (Schmidt, 1990 and unpublished); thus the two species analyzed here are among the less lethal of the analyzed species in this large and diverse group. Although not measured by identical techniques, the venom hemolytic and phospholipase activities of

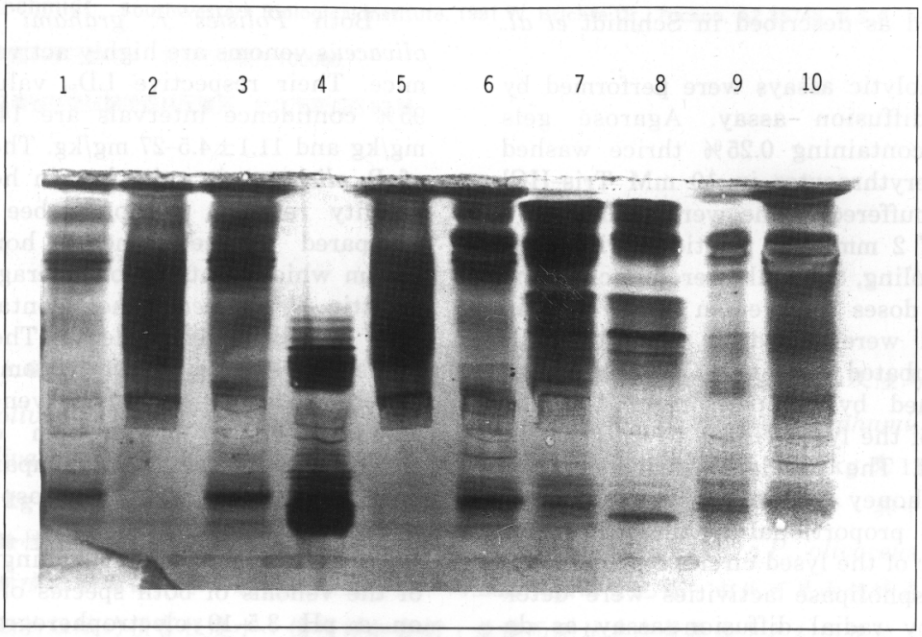

Fig. 1 Banding pattern of wasp and bee venoms on a pH 3.5-10 electropherogram. 1 = Russian bee venom Sample 2; 2 = Zhejiang bee venom; 3 = Russian bee venom Sample 2; 4 = Russian "apiotoxin"; 5 = Zhejiang bee venom; 6 = Russian bee venom Sample 1; 7 = *Polistes rothneyi grahami* venom; 8 = *Polistes olivaceus* venom; 9 = Africanized bee venom; 10 = Arizona bee venom.

P. r. grahami and *P. olivaceus* appear lower than that of *P. infuscatus* (Schmidt *et al.*, 1986). We are aware of no published electropherograms of a *Polistes* species, thus comparisons are not possible.

The collection technique used to collect the venom appears to provide a good product, that after filtration through a 0.45 micron filter yields an active fractions. That venom is suitable for a variety of pharmacological uses. The finding that the venom is generally less active in terms of hemolytic and phospholipase activities as well as mouse lethality might be an advantage. It is quite possible that this venom could have interesting active and potentially useful components without some of the highly toxic or damaging potentials of other *Polistes* preparations. Further collection and analyses are needed to determine more precise activities and potentials of these interesting venoms.

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