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# Biochemical and haematological studies of some solitary and social bee venoms

**Samy Zalat*, Zohour Nabil, Aida Hussein and Miran Rakha**

Department of Zoology, Faculty of Science, Suez Canal University, Ismailia, Egypt.

## ABSTRACT

The protein pattern of the venoms of both *Apis mellifera* and *Anthophora pauperata* has been analysed using SDS-PAGE to clarify the structure and the degree of similarity of solitary bee venom to the most known social bee venom (*Apis mellifera*). The data showed that *Apis mellifera* venom contains eleven bands with molecular weights ranging from 108,000 to 2,000D while *Anthophora pauperata* venom contains eighteen bands with molecular weights ranging from 108,000 to 6,000D. Venoms of both species showed strong similarities sharing bands with molecular weights 108,000, 93,000, 49,000, 45,000, 8,000 and 6,000D. *Anthophora pauperata* venom is characterized by a number of bands with molecular weights 37,000, 32,000, 28,000, 25,000, 20,000, 17,000, 13,000 and 10,000D. While venom of *Apis mellifera* showed two unique bands with molecular weights 3,000 and 2,000D. The effect of the venom of these two species in addition to *Bombus morrisoni* venom on the blood indices [red blood cells count (RBCs), haemoglobin content (Hb), haematocrit value (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC)] and erythrocyte osmotic fragility (EOF) showed that there is no significant difference in the blood parameters measured. Whereas, a highly significant decrease in the plasma albumin level was determined in all bee venoms studied.

**KEYWORDS:** *Apis*, *Anthophora*, *Bombus*, venom structure, haematology

## INTRODUCTION

### Honey bee venom

investigations on honey bee venom started almost a hundred years ago by Langer (1897) who found out that it consists of active and hemolytic basic components. The venom of the honey bee is a complex mixture of enzymes, polypeptides and low molecular weight components. A number of enzymes have been reported, such as phospholipase $A_2$ and hyaluronidase (Habermann & Neumann 1957), acid phosphomonoesterase and esterase

* Address for correspondence

(Benton 1967), ∝-D-glucosidase (Shkenderov *et al.* 1979), lysophospholipase (Ivanova & Shkenderov 1982), ∝-galactosidase and ∝-acetylaminodeoxyglucosidase (Hsiang & Elliott 1975) arylamidases (Bousquet *et al.* 1979). About fifty percent of the dry venom of bees is melittin, a peptide consisting of twenty-six amino acids, free of sulphur. Melittin is responsible for the pain involved in bee stings. In cell membranes, melittin forms tetrameric pores that facilitate ion diffusion (Shipolini 1984). Melittin also binds to components of skin to form a tight complex (Shipman & Cole 1972). This complex formation appears to be at least somewhat specific, as melittin does not form a complex with albumin. O'Connor & Peck (1978) mentioned that the melittin family exhibits pronounced surfactant properties and potent haemolytic activity. Secapin and tertiapin were also identified in honey bee venom (Gauldi *et al.* 1976). The biological activity of tertiapin is limited and the peptide is not particularly toxic, the $LD_{50}$ in mice being in excess of 40mg/kg; it degranulates rat peritoneal mast cells. A protease inhibitor was first reported by Shkenderov (1973), which acts to protect certain venom components from the action of proteolytic enzymes (O'Connor & Peck 1978). Nelson & O'Connor (1968) identified two or three homologous peptides with histamine present as the C-terminal residue: Nelson's peptide, procamine A and procamine B.

Histamine is the most common biogenic low molecular weight amine in Hymenoptera venoms. Markovic & Rexova (1963) noticed the presence of histamine in bee venom. The catecholamines (dopamine and noradrenalin) were identified in the venom reservoir of honey bee by fluorescence microscopy but no catecholamines could be detected in commercial samples of venom (Banks & Shipolini 1986). Nakajima *et al.* (1983) noticed the presence of putrescine, spermidine and spermine in the venom of honey bee. Cardiopep was isolated by Vick *et al.* (1974), it comprises 0.7% of honey bee venom. Moreover, amino acids, phospholipids and carbohydrates are present in bee venoms in similar quantities as in their haemolymph (Shipolini 1984). Other peptides are found in bee venoms in small concentrations only and probably do not account for much of their toxic action.

The main lethal factors of bee venoms for mammals were considered to be phospholipase A2 ($PLA_2$), melittin and apamin which are present in the venom in quantities of about 15-20%, 40-60% and 2% respectively (Habermann 1971; Banks & Shipolini 1986). Melittin was found to be the main lethal component in honey bee venom which accounts for local and general toxicity (Beard 1963; Schmidt 1995). Moreover, it was reported that melittin and $PLA_2$ interact with lipid membranes and consequently have a haemolytic action (Habermann 1971; Marsh & Whaler 1980; Schmidt 1995). They are also referred as neurotoxins as they are toxic to neuromusclar junctions (Tu 1977; Hawgood *et al.* 1988). The relationship between structure and hemolytic action was extensively studied by Schroder *et al.* (1971) using synthetic peptides. Heparin inhibits the hemolytic action of bee venom (Sergeeva 1974). This is believed to be due to the complex formation of heparin with the hemolytic component of bee venom. Cole & Shipman (1969) incubated mouse bone marrow cells with *Apis mellifera* venom or its components, and then assayed for the ability to form hemopoietic splenic colonies when transfused into lethally X-irradiated recipient mice. Colony-forming ability was annulled or decreased by venom, melittin, and phopholipase $A_2$. These workers therefore suggested that the cellular toxicity is mediated via effects at the cell surface. Blood glucose level is increased when bee venom is subcutaneously

injected into rabbits, the venom may stimulate adrenaline secretion in the animals and thereby increase glyconeogenesis (Artemov *et al.* 1972).

Although it was reported that the primary cause of death in honey bee envenomation is cardiac failure (Tu 1977; Marsh & Whaler 1980; Meier & White 1995), the mode of action of the whole honey bee venom is uncertain. Many investigators tried to block the lethality of honey bee venom and found that antisera do not readily accomplish this feat (Schmidt 1995).

**Bumble bee venom**

Early reports on the venom components of bumble-bees were reported by Welsh & Batty (1963), who found very small amounts of serotonin in extracts of the whole venom apparatus of unidentified *Bombus* species. It is not certain if this serotonin is a venom component (Piek 1986). O'Connor *et al.* (1964) and Mello (1970) found proteins in the venoms of *Bombus huntii*, *occidentalis* and *Bombus atratus*. Hoffman (1982) reported induction of allergy by stings of bumble bees. Nakajima (1979) has found putrescine in the venom of *Bombus ignitus*, and Piek *et al.* (1983) have found nearly 30 acetylcholine per venom reservoir in venom reservoir extracts as well as in dilutions of venom droplets collected from the tip of the sting of *Bombus terrestris*. The identification of the cholenergic factor with acetylcholine was confirmed using radioimmunoassay of acetylcholine. The venoms of *Bombus terrestris* and *Bombus lapidarius* also contain a component that causes slow contraction of the guinea pig ileum and the rat diaphragm (Piek *et al.* 1983). Argiolas & Pisano (1985) have found five structurally related heptadecapeptides in the venom of the bumble-bee *Megabombus pennsylvanicus* . They have named them bombolitin I, II, III, IV and V. Bombolitins lyse erythrocytes and liposomes, release histamine from rat peritoneal mast cells and stimulate phopholipase $A_2$ from different sources. Bombolitin V is as potent as melittin from honey-bee venom in lysing guinea pig erythrocytes ($ED_{50}$= 0.7 µg/ml). The bombolitins represent a unique structural class of peptides and have the same biological properties as melittin (from honey-bees). The fact that these different peptides have the same biological properties may be caused by their amphiphilic nature, a property that these peptides have in common.

**Solitary bee venom**

The venom of the solitary bee (*Xylocopa violacea*) was first described by Bert (1865), who observed that the sting of these bees could kill small birds within a few hours. In humans the sting of *Xylocopa violacea* (Hardouin 1948) and that of *Xylocopa virginica* (Hermann & Mullen 1974) seems to be extremely painful. It causes paralysis and oedema. Nakajima (1979) demonstrated that the venom of *Xylocopa appendiculata* contains about 5nmol histamine, 5nmol putrescine and 2nmol spermidine per venom reservoir. Piek (1986) has found in the venom of *Xylocopa violacea* a histamine-like activity comparable to nearly 300ng of histamine per venom reservoir and in the much smaller species *Xylocopa canescens* nearly 40ng per venom reservoir.

As mentioned before, most studies have concentrated on the venom of the common honey bee, *Apis mellifera*, and there is therefore a great gap in our knowledge concerning the venom of other Apidae. The aim of the study to investigate the main pattern of venom of solitary bee (*Anthophora pauperata*) which has not been studied

before. Moreover, the haemolytic activity of the venoms of *Apis mellifera*, *Bombus morrisoni* and *Anthophora pauperata* is discussed.

## MATERIALS AND METHODS

### Bees and venom collection

Honey bee (*Apis mellifera*) was collected from the bee culture of the Faculty of Agriculture, Suez Canal University. The bumble bee (*Bombus morrisoni*) was collected by Dr. Justin Schmidt (USA).The solitary bee (*Anthophora pauperata*) was collected from St. Katherine, Sinai, Egypt. Pure venom was obtained by the method of Schmidt (1986) and stored at -20°C.

### Electrophoresis technique

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1970). The standard curve was based on bovine serum albumin. Molecular weights of proteins were estimated following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 15-20% poly-acrylamide). Molecular weight markers (29,000-205,000D) were used as standards. Tentative identification of the substances in venoms was done by comparing the gel with those from the literature, mainly using estimates of molecular weights. Proteins were visualized with coomassie blue stain.

### Haematological and biochemical techniques

Blood was collected from 5 rats (4ml/rat) and placed into tubes containing EDTA. The whole blood of each rat was then divided into four test tubes (1ml/tube). Blood in the first tube was incubated with saline (100µl/ml) at 37°C in a water bath for 2 hours and served as a control group. The other three tubes were incubated in the same conditions as the first tube with the bee venoms (100µl/ml) at a final concentration of 1µg/ml for each bee species. Aliquots were removed after 2 hours of incubation and assayed for the following haematological parameters: Red blood cell count (RBCs) was done by visual means on Neubauer improved chamber. Haemoglobin content (Hb) was estimated using a colorimeter (Corning 252) at a wave length 546 nm using the cyanmethaemoglobin method as recommended by Drabkin & Austin (1932). Hb kit was purchased from Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany. Haematocrit value (HCT) was measured by the micro-method with capillary tubes and centrifugation force of 12000 rpm for 4 min. Mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated from the above measured parameters to serve as absolute values. Plasma was obtained after centrifugation at 4000 rpm for 15 min and stored at -20°C until assayed for the biochemical test. Biochemical assay of plasma albumin level was measured using the bromocresol green method (BCG) using a double-beam spectrophotometer ($RA_{50}$) at a wave length 628 nm. Albumin kit was purchased from Aems Company, USA.

### Erythrocyte osmotic fragility (EOF) test

Blood was collected from 5 rats (4ml/rat) and placed into tubes containing EDTA. The whole blood of each rat was then divided into four tubes (1ml/tube). Blood in the first tube was incubated with saline (100µl/ml) at 37°C in a water bath for 2 hours and

served as a control group. The other three tubes were incubated in the same conditions as the first tube with bee venoms (100 µl/ml) at a final concentration of (1 µg/ml) for each bee species. Aliquots were removed at 30, 60, 90 and 120 min for EOF determination according to the method described by Dacie & Lewis (1994).

### Statistical analysis

The results were represented as mean ± standard error. Changes in the values of haematological and biochemical parameters were analysed using student's unpaired t-test ($p<0.05$ and $p<0.001$) according to the method described by Snedecor (1956).

## RESULTS

### Electrophoretic pattern of both *Apis mellifera* and *Anthophora pauperata* venoms

The protein pattern of the venoms of both *Apis mellifera* (as a representative of social bees) and *Anthophora pauperata* (as a representative of solitary bees) were analysed using SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Tentative identification of the bands was mainly assigned on basis of molecular weights only but further studies are needed to test the identity of these molecules. This venom analysis was used to shed light upon the correlation between the venom composition and its effect.

For *Apis mellifera* venom (Figure 1 & Table 1), eleven bands were obtained with molecular weights ranging from 108,000 to 2,000D. The bands with molecular weights 108,000 and 101,000 are very high molecular weight proteins and not known. The band with molecular weight 93,000 is probably a form of phosphatase enzyme. The band with molecular weight 81,000 could be acid phosphomonoesterase. The band with molecular weight 56,000 might be hyaluronidase. The bands with molecular weights 49,000 and 45,000 could be phosphatase or forms of hyaluronidase. The band with molecular weight 8,000 might be phospholipase A or protease inhibitor. The band with molecular weight 6,000 might be minimine. The band with molecular weight 3,000 could be melittin or MCD-peptide or secapin. The band with molecular weight 2,000 might be apamin or tertiapin.

For *Anthophora pauperata* venom (Figure 1 & Table 2), eighteen bands have been detected with molecular weights ranging from 108,000 to 6,000D. The band with molecular weight 108,000 is a very high molecular weight protein and not known. The band with molecular weight 93,000 is possibly a form of phosphatase enzyme. The bands with molecular weights 87,000 and 70,000 might be forms of acid phosphomonoesterase. The bands with molecular weights 63,000 and 54,000 could be forms of hyaluronidase. The bands with molecular weights 49,000 and 45,000 could be phosphatase or forms of hyaluronidase. The bands with molecular weights 37,000 and 32,000 could be either forms of hyaluronidase or phospholipase A. The band with molecular weight 28,000 might be hyaluronidase. The band with molecular weight 25,000 could be lysophospholipase (phospholipase B) or antigen 5. The band with molecular weight 20,000 could be either phospholipase A or lysophospholipase or antigen 5 while the band with molecular weight 17,000 could be phospholipase A or lysophospholipase. The bands with molecular weights 13,000 and 10,000 might be phospholipase A or mellittin (tetramer). The band with molecular weight 8,000 might be

phospholipase A or protease inhibitor. The band with molecular weight 6,000 could be minimine.

Venoms of both *Apis mellifera* and *Anthophora pauperata* showed strong similarities (Figure 1), where they share the bands with molecular weights 108,000, 93,000, 49,000, 45,000, 8,000 and 6,000D which might be phosphatase, hyaluronidase, phospholipase A, protease inhibitor or minimine.

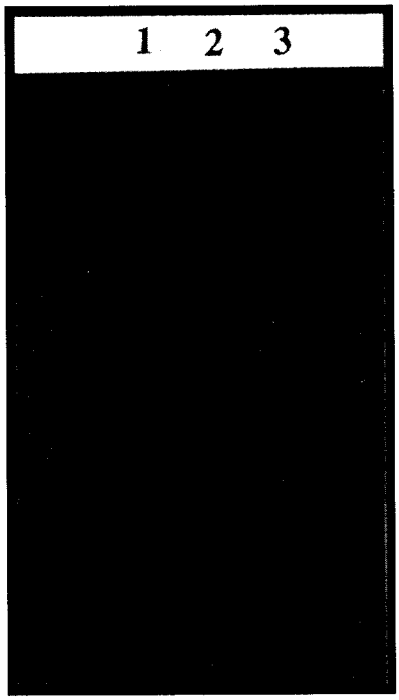


**Figure 1:** Electrophoretic patterns of the venoms of: 1, *Apis mellifera*; 2, *Anthophora pauperata* and 3, standard marker (from top to bottom: 205,000; 116,000; 97,400; 66,000; 45,000; 29,000).

**Table 1:** Analysis of the venom of *Apis mellifera*

| *Mol. Wt.* | **Comment** |
|---|---|
| 108,000 | Very high molecular weight proteins and not known. |
| 101,000 | |
| 93,000 | Probably a form of phosphatase. |
| 81,000 | Could be acid phosphomonoesterase. |
| 56,000 | Might be hyaluronidase. |
| 49,000 | Could be phosphatase or forms of hyaluronidase. |
| 45,000 | |
| 8,000 | Might be phospholipase A or protease inhibitor. |
| 6,000 | Might be minimine. |
| 3,000 | Could be melittin or MCD-peptide or secapin. |
| 2,000 | Might be apamin or tertiapin. |

**Table 2:** Analysis of the venom of *Anthophora pauperata*

| Mol. Wt. | Comment |
|---|---|
| 108,000 | Very high molecular weight protein and not known. |
| 93,000 | Possibly a form of phosphatase. |
| 87,000 | Might be forms of acid phosphomonoesterase. |
| 70,000 | |
| 63,000 | Could be forms of hyaluronidase. |
| 54,000 | |
| 49,000 | Could be phosphatase or forms of hyaluronidase. |
| 45,000 | |
| 37,000 | Could be either forms of hyaluronidase or |
| 32,000 | Phospolipase A. |
| 28,000 | Might be hyaluronidase. |
| 25,000 | Could be lysophospholipase or antigen 5. |
| 20,000 | Could be either phospholipase A or lysophospholipase or antigen 5. |
| 17,000 | Could be phospholipase A or lysophosholipase. |
| 13,000 | |
| 10,000 | Might be phospholipase A or melittin (tetramer). |
| 8,000 | Might be phospholipase A or protease inhibitor. |
| 6,000 | Could be minimine. |

## Effect of bee venoms on rat erythrocytes

Effects of *Apis mellifera*, *Bombus morrisoni* and *Anthophora pauperata* venoms on some haematological parameters such as red blood cell count (RBCs), haemoglobin content (Hb), haematocrit value (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were studied. Erythrocyte osmotic fragility (EOF) as well as plasma albumin level were also determined after incubation of rat blood with each bee venom. Table (3) represents the effect of incubation of rat blood with 1 μg/ml of each bee venom solution at 37°C for 2 hours on the above mentioned haematological parameters. As seen in the table, there is no significant difference in the all blood parameters including EOF (Fig. 2).

Figure (3) demonstrates the effect of incubation of rat blood with 1 μg/ml of each bee venom solution at 37°C for 2 hours on the plasma albumin level. It was noticed that plasma albumin level decreased significantly as a result of bee venoms application.

**Table 3:** Effect of incubation of rat blood with 1μg/ml of *Apis mellifera, Bombus morrisoni* and *Anthophora pauperata* venom solutions at 37°C for 2 hours on some haematological parameters. [Values represent means ± S.E. (n =5/ group). Non significant change from control value, student's unpaired t-test (p>0.05)].

| Parameter | Control | *A. mellifera* | *B. morrisoni* | *A. pauperata* |
|---|---|---|---|---|
| $RBC_s$(x $10^6$/$mm^3$) | 8.08±1.1 | 7.27±0.7 | 6.96±0.8 | 7.62±0.7 |
| Hb (gm%) | 6.99±0.5 | 8.16±0.8 | 7.50±0.6 | 7.35±0.6 |
| HCT (%) | 40.10±1.2 | 40.80±6.5 | 40.50±0.9 | 40.30±1.1 |
| MCV ($\mu^3$) | 53.26±6.4 | 58.45±6.5 | 61.34±7.2 | 54.78±5.0 |
| MCH (p.g) | 9.38±1.5 | 11.86±2.2 | 11.34±1.6 | 9.98±1.2 |
| MCHC (%) | 17.49±1.3 | 20.04±1.9 | 18.58±1.7 | 18.33±1.6 |

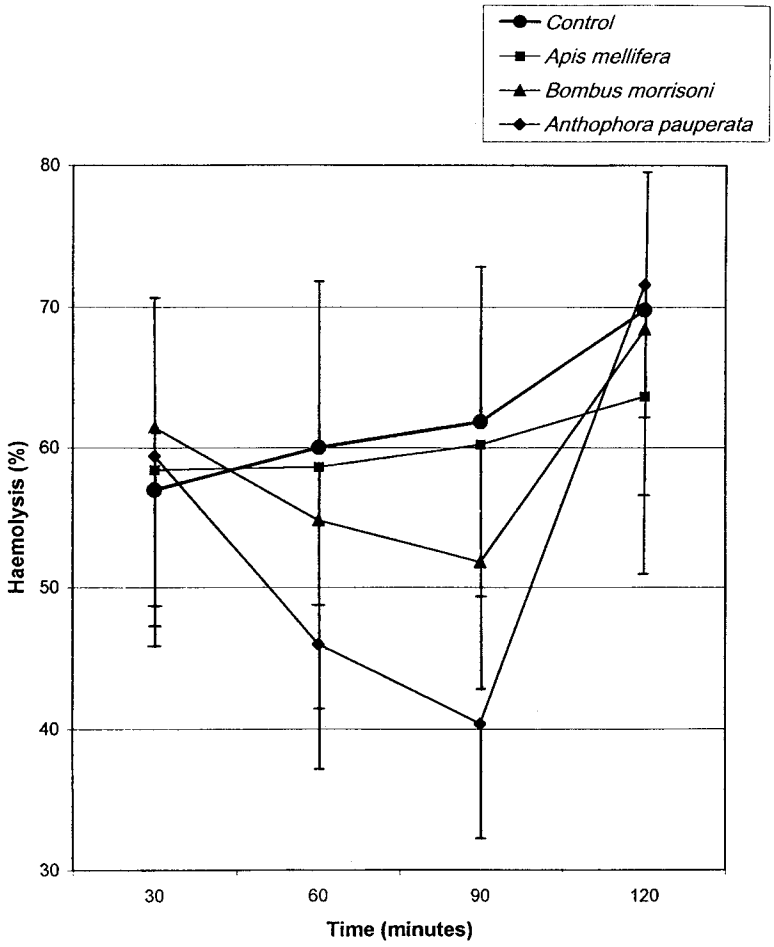


**Figure 2:** Effect of incubation of rat blood with 1 μ g/ml of *Apis mellifera, Bombus morrisoni* & *Anthophora pauperata* venom solutions at 37°C for 2 hours on erythrocyte osmtic fragility (EOF). Values represent means ± se (n =5/ group). Non significant change from control value, student's unpaired t-test (p>0.05).

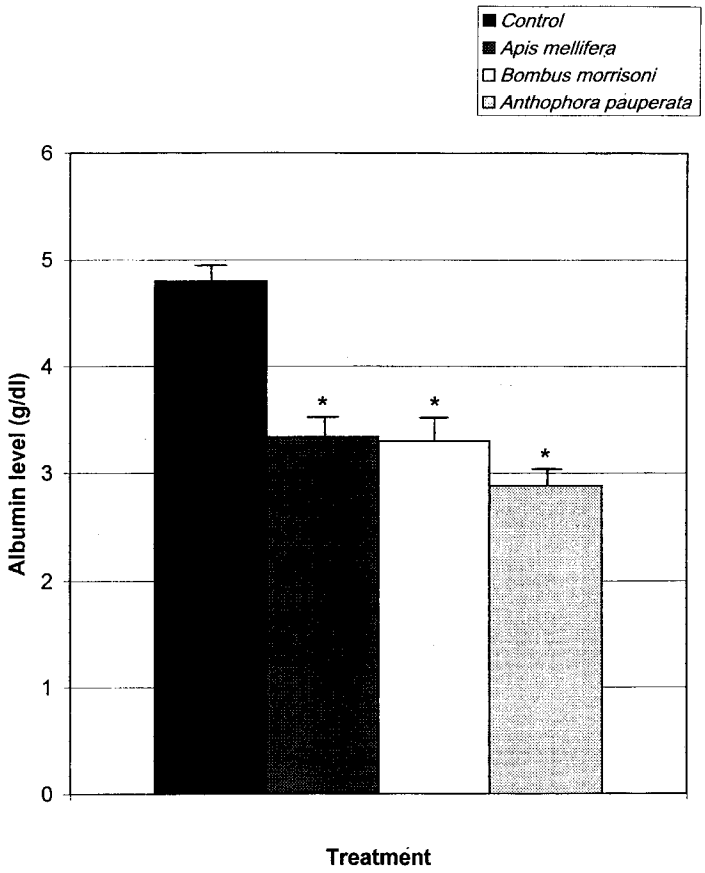


**Figure 3:** Effect of incubation of rat blood with 1 μ g/ml of *Apis mellifera*, *Bombus morrisoni* & *Anthophora pauperata* venom solutions at 37°C for 2 hours on plasma albumin level. Values represent means ± se (n =5/ group). Significantly different from control value, unpaired t-test ($p<0.001$).

## DISCUSSION

The crude venoms of the honey bee (*Apis mellifera*), the bumble bee (*Bombus morrisoni*) and the solitary bee (*Anthophora pauperata*) were used for conducting this study, since when one is envenomated it is the intact venom which is the toxic agent rather than individual components and therefore, that is worth looking at the whole venom in such a model.

Although investigations on the bee venoms started a long time ago, yet the mode of action is still uncertain. The authors conducted several studies on the venom of the same species to reveal the mechanism of toxicity induced to the myocardium as well as skeletal and smooth muscles (Nabil *et al.* 1998; Hussein *et al.* 1999; Rakha *et al.* 1999).

Venom protein analysis is a rich source of information on the biological function of the insect venom. For example, low molecular weight basic proteins or polypeptides possessing cytolytic or pain-inducing activity suggest a defensive role of the venom apparatus, such as in the case of bee venoms (apamin, bombolitins, melittin, phospholipase $A_2$) (Blum 1981; Banks & Shipolini 1986; Piek 1986).

The comparison of the electrophoretic patterns of the venoms of both *Apis mellifera* (as a representative of social bees) and *Anthophora pauperata* (as a representative of solitary bees) reveals strong similarities between them where they

share the bands with molecular weights 108,000, 93,000, 49,000, 45,000, 8,000 and 6,000D. These bands could be various forms of phospholipase A, hyaluronidase, phosphatase, protease inhibitor or minimine.

The molecular weight of one form of phospholipase A enzyme varies between 37,000 (King *et al.* 1985), 35,000 (Muller *et al.* 1981) and 38,500 (Banks & Shipolini 1986), this difference is due to the binding with carbohydrates (Shipolini *et al.* 1971). These forms of phospholipase A might therefore be found in *Anthophora pauperata* venom. Other forms of phospholipase A were reported with molecular weights 15,000 (Shipolini *et al.* 1971), 18,000-19,000 (Munjal & Elliott 1972) and 10,000 (Jentsch & Dielenberg 1972). These may be present in both *Apis mellifera* and *Anthophora pauperata* venoms. For hyaluronidase enzyme, the molecular weights reported are 50,000 (King *et al.* 1976), 45,000 (Muller *et al.* 1981), 37,000 (Kemeny *et al.* 1981), 38,000 (Shkenderov *et al.* 1979), 53,000 (Hoffman *et al.* 1977), and finally between 30,000-60,000 (Dimitrov & Natchev 1977). Such bands exist on the gel, and therefore these forms may be found in both *Apis mellifera* and *Anthophora pauperata* venoms. The molecular weight of phosphatase enzyme as judged by SDS gel electrophoresis are about 45,000 and 90,000 (Banks & Shipolini 1986) and 49,000 (Schmidt 1982). Bands corresponding to these molecular weight were also found in both *Apis mellifera* and *Anthophora pauperata* venoms. For antigen 5, the molecular weights mentioned are 25,000 (Muller *et al.* 1981) and 22,000-23,000 (King *et al.* 1983). This component probably exists in *Anthophora pauperata* venom. For lysophospholipase (phospholipase B), the only molecular weight mentioned is 22,000 ± 2,000 (Banks & Shipolini 1986). This enzyme may therefore also be found in *Anthophora pauperata* venom. Protease inhibitor has a molecular weight of 8,000-10,000 (Shkenderov 1973). With this range of molecular weight, it could be found in both *Apis mellifera* and *Anthophora pauperata* venoms. For melittin, the only molecular weight mentioned is 2,840 or 12,500 for melittin tetramer (Habermann 1972; Shipolini 1984; Banks & Shipolini 1986) which might be found in *Anthophora pauperata* venom. Lowy *et al.* (1971) isolated a very interesting peptide called minimine, which has a molecular weight of 6,000. A peptide of this molecular weight was found in both *Apis mellifera* and *Anthophora pauperata* venoms. The molecular weight mentioned for both mast cell degranulating peptide (MCD) and secapin is 2,600 (Shipolini 1984; Banks & Shipolini 1986). These components could be found in *Apis mellifera* venom. Apamin and tertiapin have a molecular weight of 2,000 (Shipolini 1984; Banks & Shipolini 1986). Peptides corresponding to these molecular weights could also be found in *Apis mellifera* venom.

It has been known that bee venom has a haemolytic action. The two haemolytic components in the honey bee venom are phospholipase $A_2$, which gives indirect action, and melittin, which is a basic peptide and provides direct lytic action (Tu 1977). A marked synergism on erythrocyte membrane with phospholipase $A_2$ and melittin has been demonstrated (Vogt *et al.* 1970; Mollay & Kreil 1974). Actually melittin forms a complex with lecithins (Mollay & Kreil 1973), suggesting that a complex may be an intermediate step in haemolysis.

Although investigations on the effect of bee venoms on the erythrocytes started a long time ago, all the previous studies were carried out on the washed erythrocytes only, not on whole blood, since Habermann & Neumann (1954) suggested that melittin from the honey bee haemolyses serum-free erythrocytes by a direct action that makes the cell membrane permeable to haemoglobin but does not destroy the cell. In the present study,

the action of crude venoms of the honey bee (*Apis mellifera*), the bumble bee (*Bombus morrisoni*) and the solitary bee (*Anthophora pauperata*) on rat whole blood was studied in order to determine their ability to induce red blood cell haemolysis.

The effect of bee venoms on some haematological parameters showed that the changes in blood parameters were statistically non significant indicating that red blood cell haemolysis induced by bee venoms was very limited which leads to the suggestion that there is a certain mechanism of protection against bee venoms induced haemolysis of red blood cells. A non-significant decrease in the erythrocyte osmotic fragility (EOF) as a result of bee venoms was also noticed assuring that there is a protective mechanism against bee venoms toxicity on the erythrocytes.

In an attempt to reveal the mechanism of protection against haemolysis of erythrocytes induced by bee venoms, plasma albumin level was measured. It was noticed that plasma albumin level decreased significantly as a result of bee venoms application indicating that plasma albumin inhibits the ability of bee venoms to induce red blood cell haemolysis. This might be achieved by forming a precipitating complex with the direct lytic factor (DLF) in bee venoms. This idea is in agreement with Azhitskii *et al.* (1995), who observed that bee venom melittin interacts with human blood albumin and forms a precipitating complex. Franklin & Baer (1974) observed that melittin can interact with a serum component to form an insoluble complex. Dirks & Sternburg (1972) also found that the venom of *Apis mellifera* is capable of complexing with normal rabbit serum. The present data agrees with Rudenko & Nipot (1996), who observed that albumin and some other agents inhibit melittin-induced haemolysis of erythrocytes and suggested that the protective mechanism may be due to the existence of melittin-specific membrane inhibitory components (MICs), which initially protect the cell against the lytic action of peptides. A model of melittin-induced haemolysis is proposed according to which features of haemolysis are determined by consecutive stages of peptide-membrane interactions and depend on whether or not an anti-lytic triple complex, including a membrane inhibitory component, an inhibitor and the peptide is formed.

Accordingly, it can be concluded that plasma albumin inhibits the ability of bee venoms to induce red blood cell haemolysis. This might be achieved by forming a precipitating complex with melittin in the honey bees venom or bombolitins in the bumble bees venom, since Argiolas & Pisano (1985) have found five structurally related heptadecapeptides in the venom of the bumble bee (*Megabombus pennsylvanicus*). They have named them bombolitins and suggested that they have the same biological properties as melittin (from honey bee venom) and they lyse erythrocytes. Moreover, this complex might be formed with a melittin-like substance in the solitary bees venom, since Piek (1986) reported that there is a melittin-like substance in the venom of the solitary bee (*Xylocopa violacea*).

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## الملخص العربى

# دراسات بيوكيميائية وهيماتولوجية على سموم بعض أنواع النحل الإنفرادى والإجتماعى

**سامى زلط، زهور نبيل، عايدة حسين و ميران رخا**

قسم علم الحيوان – كلية العلوم – جامعة قناة السويس – الإسماعيلية – مصر

تناولت الدراسة التحليل البروتينى لسم كل من نحل العسل (*أبيس ميلليفرا*) والنحل الإنفرادى (*أنثوفورا بيبوريانا*) لمعرفة التركيب ومدى التشابه بين سم كلا منهما. أوضحت الدراسة أن سم نحل العسل يحتوى على ١١ مركب بروتينى تتراوح أوزانهم الجزيئية بين ١٠٨,٠٠٠–٢,٠٠٠ دالتون، بينما يحتوى سم النحل الإنفرادى على ١٨ مركب تتراوح أوزانهم الجزيئية بين ١٠٨,٠٠٠ – ٢,٠٠٠ دالتون. أثبتت النتائج أن هناك تشابه كبير بين تركيب سم كل من نحل العسل والنحل الإنفرادى (*أنثوفورا بيبوريانا*) وذلك بناء على وجود المركبات البروتينية ذات الأوزان الجزيئية: ١٠٨,٠٠٠ ، ٩٣,٠٠٠، ٤٩,٠٠٠ ، ٤٥, ٠٠٠، ٨,٠٠٠، ٦,٠٠٠ دالتون. تميز سم نحل العسل بوجود المركبات ذات الأوزان الجزيئية الصغيرة وهى ٠٠٠ ,٣، ٠٠٠ ,٢ دالتون بينما تميز سم النحل الإنفرادى (*أنثوفورا بيبوريانا*) بوجود المركبات ذات الأوزان الجزيئية ٠٠٠ ,٣٧، ٠٠٠ ,٣٢، ٠٠٠ ,٢٨، ٢٥,٠٠٠ ٠٠٠ ,٢٠، ٠٠٠ ,١٧، ٠٠٠ ,١٣، ٠٠٠ ,١٠ دالتون والتى لم تمثل فى سم نحل العسل.

أوضحت الدراسة تأثير هذه السموم بالإضافة إلى سم النحل الطنان (*بومبيس مورسونى*) على معايير الدم المختلفة ومعدل الهشاشة الأسموزية لكرات الدم الحمراء ووضح أن التغيرات الناتجة فى جميع المعايير السابقة كانت غير معنوية بينما إنخفض مستوى الزلال فى البلازما بشكل معنوى كبير.