

قسم : علم الحيوان .
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التأثير الفسيولوجي لسم دبور البلح على الحركة الذاتية في الفئران
وتكسيرات كرات الدم الحمراء ومحتويات البروتين الكلي والاحماض الامينية الضرورية
في سيرم الانسان
خديجة حسن

أجري البحث على ٢٤ فأرا لدراسة التأثير الفسيولوجي لسم دبور البلح على الحركة
الذاتية في الفئران .

كما تمت دراسة تأثير سم دبور البلح على تكسيرات الدم الحمراء ومحتويات
البروتين الكلي والأحماض الأمينية الضرورية في سيرم الانسان .
وقد اتضح من نتائج هذ البحث مايلي :

١- حقن سم دبور البلح بتركيزات ١ ، ٢ ، ٣ ، ٤ آله لسع / ١٠٠ جم من وزن جسم الفأر
في ذكور الفئران الناضجة أدى الى حدوث نقص معنوى ملموس في الحركة الذاتية لها في
حالة استخدام ١ ، ٢ ، ٣ آله لسع وبلغ أدنى حد له بعد ١¼ ساعة من الحقن . ثم
استمرت الحركة في الازدياد حتى عادت الى معداها الطبيعي بعد ٢٤ ساعة من الحقن .
غير أن استخدام ٤ آلات لسع أدى الى حدوث أعراض آلام ظاهرة واستثاره ثم تبعها
حدوث هبوط وموت فجائي للفئران التي حقنت بمعدل آله لسع واحده / ٢٠ جم من
وزن الجسم .

٢- هذا الى أن النتائج قد أظهرت أن سم دبور البلح لخصائص متميزة في تكسير
كرات الدم الحمراء في دم الانسان لكن سرعة تكسير هذه الكرات الدموية الحمراء كانت
أكبر في حالة السم المسخن لدرجة ٥٦°م لمدة ٣٠ دقيقة عنها في السم غير المعامل حراريا
٣- وبالإضافة الى ماتقدم فان سيرم الانسان المعامل بسم دبور البلح أحدث تناقصا
تدرجيا في محتوى البروتين الكلي حيث بلغ أقصى معدل من الانتخفاض عند تركيز ٦ آلات
لسع، وعلى العكس أحدث السم زيادة ملموسة في الأحماض الامينية الضرورية نتيجة
لنشاط انزيم البروتينيز الموجود في السم .

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**PHYSIOLOGICAL EFFECT OF WASP VENOM ON SPONTANEOUS
ACTIVITY OF ALBINO RATS AND HUMAN BLOOD HAEMOLYSIS,
TOTAL PROTEIN AND ESSENTIAL AMINO ACIDS CONTENTS
IN HUMAN SERUM**

(With 3 Tables and One Figure)

By
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SUMMARY

This investigation was performed on 24 mature male rats to clarify the physiological effect of date wasp venom on spontaneous activity of albino rats.

Moreover, the effect of date wasp venom on human blood haemolysis, total protein and essential amino acids contents were assessed.

The data led to the following conclusions:

1- Intraperitoneal injection of date wasp venom in doses of 1,2,3 and 4 stings/100g. body weight into mature male albino rats produced significant decrease in their spontaneous activity in the three former doses reaching its maximum at 1.5 hours of injection. Thereafter, the movements began to increase and became normal 24 hours after injection. However, the latter dose (4 stings/100 g. body weight) produced remarkable symptoms of pain and irritability followed by strong depression and final sudden death of the rats weighing 80 g.

2. Furthermore, the data showed that date wasp venom possessed a marked haemolytic power on human blood. However, the rate of haemolysis was rather high in case of the heated venom at 56°C for 30 minutes than that of unheated venom.

3- Wasp venom treated human serum was accompanied by a graded decrement in total proteins reaching its maximal level in the dose of 6 stings. On the contrary the essential amino acids contents were increased due to the presence of protease enzymes in the venom.

INTRODUCTION

Vespa orientalis, known in Egypt as date wasp, produces a potent venom which contains a various biologically active substances possessing many physiological effects on man and animals (ABDEL-FADIL, 1980 and SELEEM, 1981).

JOSHUA and ISHAY (1973) stated that the toxicity of protein or protein bound substances of wasp venom were attributed to haemolytic activity. Wasp venom possesses a marked haemolytic activity. FISCHL *et al.* (1972) reported that there are four haemolytic fractions of high molecular weight, which are considered either to be proteins or attached to protein.

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EDERY *et al.* (1972) and ISHAY *et al.* (1974) found that wasp venom produces a significant decrease in activity as well as a noticeable paralysis in mice and cats. However, up to the author's knowledge there are no available data on the effect of wasp venom on the essential amino acids composition. Likewise, rather limited literature on the effect of wasp venom on the interaction between protein content, haemolytic effect and activity rate in experimental animals is available. Therefore, it seemed of value to carry out the present investigation. *Vespa orientalis* has always been a source of economic trouble. It is considered to be the most dangerous enemy of the honey bees, since the wasp workers destroy the nests of the bees. This may lead to the destruction of honey resources as well as causing a lot of troubles for humans. This also emphasized the importance of carrying out the present investigation.

MATERIAL and METHODS**1- Materials :****1-1: Experimental animals :**

4 groups of mature male albino rats 6 animals each weighing 80-120 g. were used. Such groups were intraperitoneally injected with 1,2,3 and 4 stings/100 g. body weight of the rats.

1-2: Wasp venom :

A total number of 500 wasps (*Vespa orientalis*) were collected from the bee hives and their nests from Assiut University Farm in the period between June and September 1987. The stings were obtained and prepared applying the method previously recommended by HASSAN (1968). The venom concentration was 0.5 mg/ml. distilled water. This venom extract was used for haemolysis experiments. However, in another experiments the venom extract contained 5 stings 1 ml. distilled water.

1-3: Human red cell suspension :

Human red cells suspension was prepared as described by GREEN (1972) using a 0.9% sodium chloride solution (isotonic saline solution).

1-4: Human serum :

Human serum was prepared as outlined by OSER (1965).

2- Methods :**2-1: Human blood haemolysis :**

The volume of packed cells in the centrifuge tube was recorded, while the supernatant fluid was carefully removed. A suspension containing 1 cm³ red cells in 49 cm³ of saline solution was prepared. Various dilutions of venom solutions namely: 125, 62.5, 31.25, 15.62, 7.8, 4, 2, 1 and 0.5 ug were prepared in 9 test tubes each containing 0.25 ml. saline solution. The last test tube (No. 10) contained 0.25 ml. saline solution only and served as control. For each of the ten above mentioned tubes 0.25 ml. from the red blood cells suspension was added.

2-2: Evaluation of the spontaneous coordinate activities of albino rats :

Spontaneous coordinate activities of albino rats were recorded by using the Activity cage 7401 (Ugo Basile-Biological Research Apparatus-Italy) before injection (control).

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immediately after intraperitoneal injection, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12 and 24 hours after injection with the venom extract of 1, 2, 3 and 4 wasp stings/100 g. body wt.

2-3: Determination of serum total proteins :

The serum total proteins of human were determined applying the Biuret method (VARLEY, 1969).

2-4: Determination of essential amino acids :

Essential amino acids contents of human serum except tryptophan were determined qualitatively and quantitatively by thin layer chromatography applying the method described by BLOCK *et al.* (1958). Tryptophan content was determined applying the Standard chemical method described by SPIES and CHAMBERS (1949).

RESULTS and DISCUSSION

A) Spontaneous activities of albino rats :

Intraperitoneal injection of date wasp venom in doses of 1 sting, 2 stings, 3 stings and 4 stings/100 g. body weight into mature male albino rats produced significant decrease in their spontaneous activities in the three former doses, reaching its minimum at 1.5 hours after injection. thereafter, the movements began to increase and became normal 24 hours after the injection (Table 1). However, the latter dose (4 stings/100 g. body weight) produced remarkable symptoms of pain and irritability followed by strong depression and final sudden death of the rats, weighing 80 g. Such finding denoted that the lethal dose of date wasp venom was 1 sting/20 g. body weight.

Such findings are in good agreement with KAPLINSKY *et al.* (1974), who reported that arrhythmias, shock and death by multiple stings may be attributed to the presence of potent vasodilator materials with positive inotropic properties existing in the venom and contribute to its action. EDERY *et al.* (1972) stated that in vivo the wasp venom produced central and peripheral paralysis in mice and cats. The neurotoxic effect of wasp venom extract was outlined by ISHAY *et al.* (1974). Injection of the relatively small doses (1 and 2 stings/ 100 g of body weight of albino rats produced a central suppression of brain activity.

B) Human blood haemolysis :

The data revealed that haemolysis took place up to the 5th test tube in which the venom dilution was 1/32000 (i.e. titre of haemolytic power was 32000). On the contrary, the haemolysis was nonexistent in the other five tubes. (Table 2). Similar trend was recorded in duplicate experiments. Likewise, the same findings were obtained when the above-mentioned procedures were repeated with heated venom. Heating was carried out on a water bath at 56°C for 30 min. However, the rate of haemolysis was rather high incase of the heated venom at 56°C for 30 minutes than that of the unheated venom. This might denote the presence of some heat labile antihaemolytic factors. EDERY *et al.* (1972) reported that protease enzyme and the possibility of phospholipase activity is responsible for venom haemolytic action. According to FISCHL *et al.* (1972) found that pure venom and venom sac extracts of *Vespa orientalis* possess a marked haemolytic activity. There are four haemolytic fractions of high molecular weight, considered either to be proteins or attached to proteins. JOSHUA and ISHAY (1973) stated that the erythrocytes of man were haemolysed by *Vespa orientalis*, which coincides with the data obtained in this investigation. Earlier, BOESE (1950) reported that haemolysis was stronger on a suspension of red cells than of the whole blood.

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C) Total proteins and essential amino acids of human serum :

The effect of wasp venom treatment on the human serum total proteins and essential amino acids contents are represented in table 3 and Fig. 1. The data revealed that the human serum total proteins (HSTP) were 7 g/100 ml in untreated samples. However wasp venom treated serum was accompanied by a graded decrement in HSTP reaching its minimal level in wasp venom dose (6 stings). The data revealed that in general the total essential amino acid contents quantitatively varied in wasp venom treated samples, although no qualitative changes were detected (Fig. 1). All studied venom concentrations resulted in an increase in the total essential amino acids content. However, leucine-isoleucine mixture and histidine recorded highest level of increment, whereas methionine represented the least percentage. According to FISCHL *et al.* (1972) most protein fractions in wasp venom are basic proteins. The toxicity of protein or protein bound substances were attributed to haemolytic activity (JOSHUA and ISHAY, 1973). ISHAY *et al.* (1977) reported that the lethality and the toxic constituents of oriental hornet venoms is correlated to the protein contents of the venom sac extract.

The reduction that took place in the total protein content of wasp venom treated human serum might be attributed to the presence of protease enzyme in this venom. EDERY *et al.* (1972) found protease enzyme in *Vespa orientalis* venom capable of splitting wide range of substrates and responsible for venom haemolytic action. Such finding Justified the increase in the total essential amino acids content in wasp venom treated human serum. Moreover, it may be concluded that the release of amino acids due to the protease activity is accompanied by enhancing the spreading phenomena of the venom in the skin of the victim.

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Table (1): Effect of different doses of wasp venom on the spontaneous coordinate activity of mature male albino rats.

Dose (stings/100g	Before Injection	Number of spontaneous movements/5minutes.											
		Immediate	0.5h	1h	1.5h	2h	2.5h	3h	4h	6h	9h	12h	24h
1 sting/100 g	271	186	125	69	25	110	124	164	206	226	242	254	284
body weight	+8.92	±6.48	±4.71	+3.55	±0.94	±2.78	±3.27	±3.30	±3.96	±5.00	±6.24	±6.42	±7.66
t-test		1.284	2.411	3.506	4.571	2.871	2.578	1.874	1.109	0.733	0.443	0.251	0.156
2 stings/100 g	283	177	98	76	30	70	88	119	141	171	200	251	289
body weight	+8.45	+5.61	+2.59	+1.84	+0.58	+2.1	+1.64	±1.69	±2.31	±2.91	±6.62	±6.80	±8.75
t-test		1.74	3.49	3.90	4.98	4.08	3.77	3.17	3.03	2.09	0.37	0.49	0.10
3 stings/100g	300	192	132	56	29	96	119	128	155	174	196	226	295
body weight	±7.51	±5.49	±4.48	±1.24	±0.64	±1.94	±2.84	±2.69	±1.32	±3.77	±3.96	±4.88	±7.30
t-test		2.01	2.14	5.64	5.99	4.38	3.76	3.59	3.17	2.50	2.04	1.38	0.08

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Table (2): Effect of wasp venom concentration on human blood haemolysis

Tube No.	Wasp venom concentration (μg)	Occurrence of haemolysis
1	125 μg	+
2	62.5 μg	+
3	31.2 μg	+
4	15.6 μg	+
5	7.8 μg	+
6	4.0 μg	-
7	2.0 μg	-
8	1.0 μg	-
9	0.5 μg	-
10	0.0 μg (saline solution only).	-

Table (3): Effect of wasp venom concentration on total protein and essential amino acids contents in human serum.

Total protein and essential amino acids	Wasp venom concentration						
	Control	1 sting	2 stings	3 stings	4 stings	5 stings	6 stings
Total protein (g/100 ml serum)	7.0	6.8	6.5	6.4	6.0	5.9	5.8
Tryptophan	0.25	0.20	0.20	0.21	0.25	0.25	0.26
Threonine	0.31	0.32	0.32	0.34	0.29	0.32	0.34
Valine	0.49	0.62	0.62	0.49	0.49	0.65	0.67
Methionine	0.11	0.10	0.10	0.10	0.11	0.11	0.13
Phenylalanine	0.53	0.56	0.55	0.56	0.54	0.55	0.57
Lysine	0.60	0.60	0.63	0.63	0.55	0.60	0.67
Histidine	0.53	0.55	0.55	0.55	0.67	0.67	0.68
Arginine	0.25	0.24	0.26	0.27	0.25	0.29	0.36
Leucine-isoleucine mixture	0.60	0.82	0.84	0.95	0.88	0.95	1.02
Total essential amino acids (mg/100 ml serum)	3.67	4.01	4.07	4.14	4.04	4.39	4.70

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LEGEND OF FIGURES

Fig. (1): Effect of graded doses of wasp venom on the essential amino acids of human blood serum.

- | | |
|-----------------|---------------|
| 1- One sting | 2- Two stings |
| 3- Three stings | 4- Four sting |
| 5- Five stings | 6- Six stings |
| 7- Control. | |
-
- | | |
|---------------------------------|-------------------|
| A. Lysine | B. Histidine |
| C. Arginine | D. Threonine |
| E. Valine | F. Methionine |
| G. Leucine - isoleucine mixture | H. Phenylalanine. |



