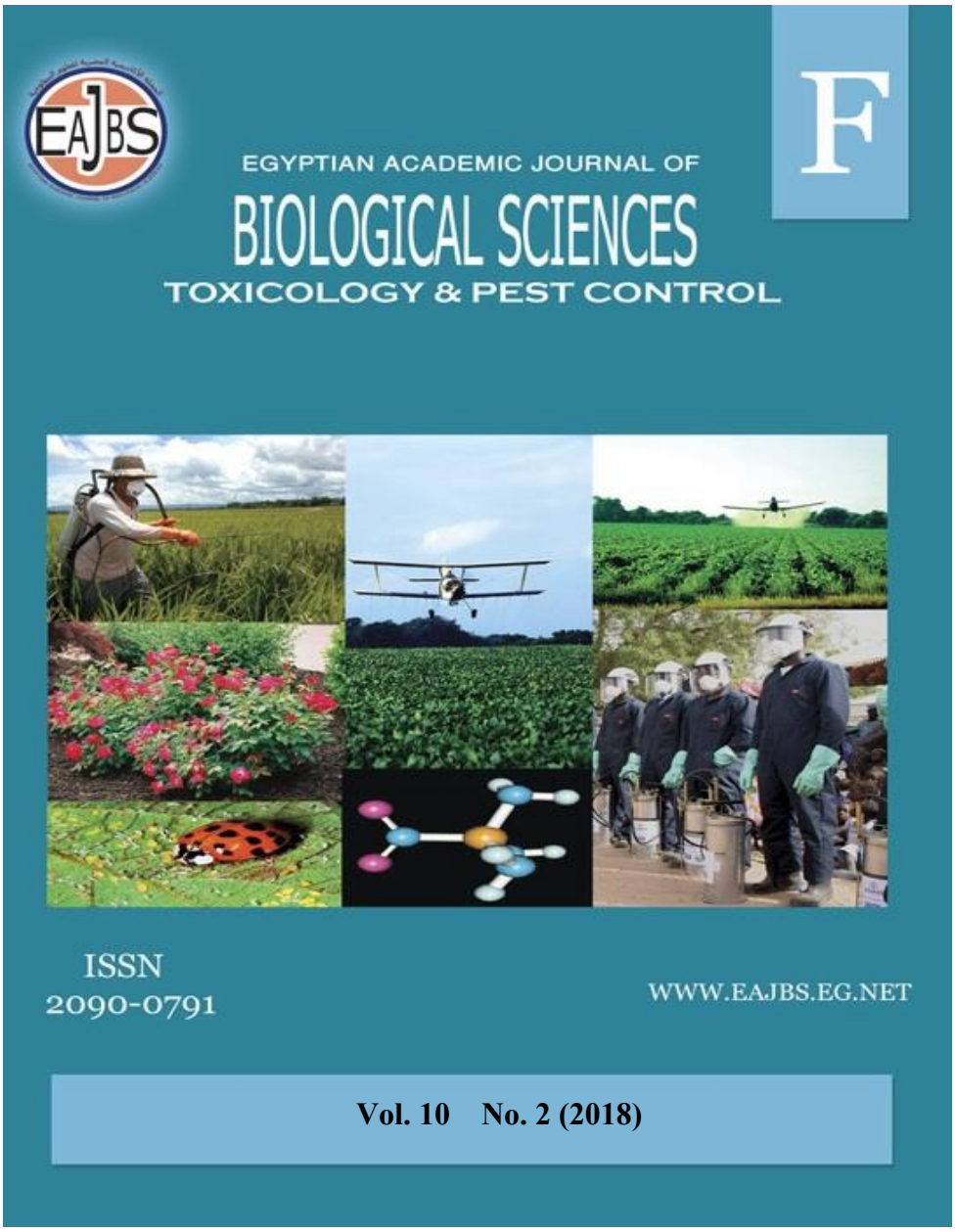

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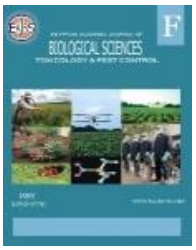

Toxic Hematological Effects of *Vespa orientalis* Venom in the Rat, *Rattus albus*

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ABSTRACT

The pharmacological properties of *Vespa orientalis* venom and its toxicity against the mammalian albino rat, *Rattus albus* was studied by direct stinging or by using the venom sac homogenate. The degree of hemolysis and other hematological changes as induced by stinging or injection the rat, *Rattus albus* with venom sac homogenate (VSH) were investigated in-vivo. The results obtained showed that both stings and venom injection had toxic effects on the blood of rats, especially the multiple stings (3 or 4 stings) and high doses of VSH (6 and 12mg/1ml saline solution). These treatments evoked a significant decreased in WBCs, RBCs, HG and HCTs but a significant increase of PLTs indicating that *Vespa orientalis* venom has a hemolytic activity on the in-vivo rat blood.

INTRODUCTION

Social wasps, belonging to the Vespidae family, are known stingers of the Hymenoptera order, and are divided into two subfamilies: Vespinae, typical of temperate areas, and Polistinae, from tropical areas (Richards, 1978). They possess highly toxic venom, rich in enzymes, biogenic amines and biologically active peptides (Habermann, 1972; Nakajima, 1986), with predominantly neurotoxic, algesic, cytotoxic, haemolytic, hemorrhagic and allergenic pharmacological activities (Ho and Ko, 1998; Mortari et al., 2005). Wasp venom is a complex biological substance producing many physiological effects when injected into experimental animals (Edery and Ishay, 1965).

Social wasps, like many other venomous animals, use their venom either to capture prey or for defense, and their venoms are able to kill small vertebrates, insects and, as a consequence of multiple stings, even large vertebrates (Piek and Spanjer, 1986). The *Vespa* wasps species are important venomous insects endangering humans, causing severe envenoming cases.

The Current study aimed to evaluate some of the pharmacological properties of *Vespa orientalis* venom by investigating the toxicity of venom sac homogenate (VSH) and stings of *Vespa orientalis* against one of mammalian animals namely; albino rat, *Rattus albus*.

MATERIALS AND METHODS

Collection of Hornets (*Vespa orientalis*):

Hornets were collected during summer seasons (July-September, 2013 & 2014) from traps settled between the honey bee nests at the Department of Honey Bee Researches, Institute of Plant Protection, Ministry of Agriculture, Dokki, Giza, Egypt. The hornet traps were placed to trap hornets as they feed on honey and workers. The collected hornets were transported to the Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt. Then, the hornets were refrigerated to keep them immobilized and thereby enhance ease of handling and dissection. When time did not allow immediate dissection, the hornets were stored frozen at -20°C.

Toxic Effects of *Vespa orientalis* Venom on Rat:

1. Experimental Animal:

Eighty-four males and females of albino rats, *Rattus albus* (6-7 weeks old, with a mean weight of 120±5 gm) were obtained from farms of experimental animals in Helwan, Egyptian company for the production of Vaccines, Sera & Drugs (EGYVAC-VACERA), Ministry of Health, Egypt. The experiments were carried out at Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt. Rats were housed and maintained in a temperature-controlled room at 27–28°C and a 12h light/dark cycle, housing period ranged from one to two weeks prior to the initial of the experiment was important to ensure their environmental adaptation. Males and females placed separate, (3 rats/plastic cage). Animal experiments were conducted according to the guidelines of Animal Care and Ethics Committee by Public Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) in accordance with the recommendations for the proper care and use of laboratory animals approved by Animal Care Committee of the National Research Centre, Egypt.

2. Hematological Effects in Rats:

a. By Direct Stinging:

In the direct stinging, newly hunted wasps were permitted to sting the rat in the abdominal region, where the wasp clutching carefully by forceps at thoracic part even sees the ovipositor penetrates the skin of the rat:

- Twelve male rats were divided into four groups (3rats/group) and twelve female rats were also divided into four groups (3rats/group) before stinging by wasps.
- Blood samples were collected under diethyl ether anesthesia from all males and females (1ml blood/rat), by capillary tubes from retro-orbital venous plexus.
- Blood was collected in EDTA (Ethylene Diamine Tetra Acetic Acid). These 24 blood samples used as negative control (not stung) groups so that clarify blood picture before stinging by wasps.
- After 24 hours from obtaining the blood from the negative control rats, they were stung by wasps under diethyl ether anesthesia was as follows; where the members of the first group exposed only to one sting. While the members of the second group exposed to two stings, the members of the third group were exposed to 3 stings and the members of the fourth group exposed to four stings.
- All rats were stung by the full sting. The stinging period to range from 20 to 30 seconds.

- After six hours from envenomation, blood samples were collected under diethyl ether anesthesia from all twelve males and twelve females (1ml blood/rat) by capillary tubes from retro-orbital venous plexus.
- The hematological parameters, white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HG), hematocrits (HCTs) and platelets (PLTs) were measured in 48 blood samples (24 blood samples before stinging of rats + 24 blood samples after stinging of rats). The hematological parameters were measured in blood collected in EDTA by using CBC Analyzer (Sino Thinker. SK 9000, U.S.A).

b. By Injection of Rats With Venom Sac Homogenate (VSH) of *Vespa orientalis*:

Preparation of the concentrations:

Three different concentrations prepared from VSH as follows:
- The first concentration (6 venom sacs soaked in 1ml saline solution).
- The second concentration (12 venom sacs soaked in 1ml saline solution).
- The third concentration (24 venom sacs soaked in 1ml saline solution).

Calculation of the concentrations:

- The 1st concentration; 6 venom sac×0.5mg (weight of venom sac) = 3mg venom.
- The 2nd concentration; 12 venom sac×0.5mg = 6 mg venom.
- The 3rd concentration; 24 venom sac×0.5mg = 12 mg venom.
- Venom sacs in three different eppendorf tubes (2ml) with saline solution were homogenized by ultra-homogenizer for ten minutes and centrifuged by cooling centrifuge at 10000 r.p.m for15 minutes at -4°C.
- The homogenate was injected in rats by the intraperitoneal (IP) method.
- Nine male rats were divided into three groups (3rats/group) and nine female rats were also divided into three groups (3rats/group) and injected with saline solution only (positive control).
- Blood samples were collected from males and females under diethyl ether anesthesia (1ml blood/rat) by capillary tubes from retro-orbital venous plexus in EDTA.
- After 24 hours from obtaining the blood from rat males the females, they were injected IP method under diethyl ether anesthesia. The injection with VSH accomplished as follows;
- Rats of the first group were injected by 3 mg/1ml saline solution.
- Rats of the second group were injected by 6 mg/1ml saline solution.
- Rats of the third group were injected by12 mg/1ml saline solution.
- After three hours from envenomation, blood samples were collected from all nine males and nine females (1ml blood/rat.) by capillary tubes from retro-orbital venous plexus.
- The hematological parameters, white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HG), hematocrit (HCTs) and platelets (PLTs) were measured in 36 blood samples (18 blood samples before injection with VSH +18 blood samples after injection). The hematological parameters were measured in blood collected in EDTA by using CBC Analyzer (Sino Thinker. Sk 9000, U.S.A).

3. Statistical Analysis:

The statistical package for the social sciences (SPSS, version 20) was used in data analysis. Data were expressed as mean ± S.D. One way analysis of variance (ANOVA) was used to compare between groups followed by Fisher's least significant difference (LSD) analysis. F-probability, obtained from one-way ANOVA, expresses the effect between the groups. P values less than 0.05 were considered significant.

RESULTS

1. Hematological Effects in Rats:

a. By Direct Stinging:

The degree of hemolysis and other Hematological changes as induced by stinging the rat, *Rattus albus* (males and females) are given in tables (1 & 2) and illustrated in figures (1-5).

As shown from the results; the stinging of male rats induced significant decrease of erythrocyte count, where the mean count of WBCs was decreased from [7.56±2.47, 10.40±0.916, 11.2±3.70 and 10.0±2.17 to 3.70±1.35, 5.23±1.28, 6.73±1.40 and 5.60±1.17] × (10^{-3}/mm^3) in rats exposed to one, two, three and four stings, respectively. The WBCs count was reduced nearly to half of the non-stung rats.

In addition, the mean count of RBCs only was significantly decreased ($P<0.05$) in rat males exposed to four stings, where it decreased from (4.99±0.70 to 3.04±1.15) × (10^{-6}/mm^3). Also, hemoglobin (HG) conc. and hematocrit (HCTs) were significantly decreased ($P<0.05$) in male rats exposed to only four stings, where the mean conc. of HG was decreased from 12.93±2.51 to 7.16±4.44 g/dl and the mean percent of HCTs was decreased from 33.50±7.59 to 20.26±11.22 %.

On the other hand, the mean count of blood platelets (PLTs) was significantly increased ($P<0.05$) from [222.33±74.88 to 305±100.70 and from 219.33±69.21 to 301.33±96.47] × (10^{-3}/mm^3) in male rats exposed to three and four stings, respectively.

As shown from the results in table (2) and figures (1-5), the Hematological changes as induced by stinging female rats; there was a significant decrease ($P<0.05$) in WBCs and RBCs counts, where the mean count of WBCs was decreased from [11.23±3.78, 13.46±1.79 and 10.70±3.70 to 5.50±1.63, 6.46±1.62 and 5.80±4.24] × (10^{-3}/mm^3) in females exposed to two, three and four stings, respectively.

The mean count of RBCs decreased from (4.91±0.12, 4.83±0.15 and 4.85±0.42 to 4.10±0.40, 3.53±0.28 and 3.25±0.75) × (10^{-6}/mm^3) in females exposed to two, three and four stings, respectively. Also, the amount of HG and HCTs percentage were significantly decreased in female rats exposed to two, or three and four stings. The HG amount was decreased from 15.50±3.83, 13.23±1.27 and 12.96±0.15 to 11.10±0.91, 9.50±0.1 and 7.40±2.43 g/dl in females exposed to two, three and four stings, respectively. While, HCTs percent was decreased from 32.66±2.19 and 32.03±0.37 to 25±1.32 and 19.76±7.19 % in rats exposed to three and four stings, respectively.

On the other hand, the mean count of PLTs was significantly increased ($P<0.05$) in females exposed to three and four stings. It increased from [155±60.91 to 171.33±79.82 and from 173.33±50.85 to 226±80.13] × (10^{-3}/mm^3) in females exposed to three and four stings, respectively.

From the aforementioned results, it has appeared that the number of stings that rats (males & females) being exposed to them, the count of WBCs, RBCs, HG conc. and HCTs % were decreased, while PLTs was increased. Moreover, the statistical analysis of data indicated no significant difference between male and female rats in the Hematological changes tested.

Table (1): Hematological changes in *Rattus albus* males as induced by *Vespa orientalis* stings

Groups and stings / Hematological parameters	Untreated groups (Mean ± SD)				Treated groups (Mean ± SD)			
	(1)	(2)	(3)	(4)	One sting (1)	Two stings (2)	Three stings (3)	Four stings (4)
WBCs	7.56±2.47	10.40±0.916	11.2±3.70	10±2.17	3.70±1.35*	5.23±1.28**	6.73±1.40*	5.60±1.17*
RBCs	4.73±0.67	4.93±0.63	4.81±0.54	4.99±0.70	4.57±0.63^{NS}	4.39±0.78^{NS}	3.83±0.97^{NS}	3.04±1.15**
HG	12.73±1.90	12.93±1.23	13.40±1.50	12.93±2.51	12.03±1.70^{NS}	11.26±2.11^{NS}	9.36±3.40^{NS}	7.16±4.44*
HCTs	32.23±1.98	32.46±4.03	34.43±4.07	33.50±7.59	31.73±4.14^{NS}	29.76±7.30^{NS}	27.23±8.80^{NS}	20.26±11.22*
PLTs	217.66±68.87	270.66±18.14	222.33±74.88	219.33±69.21	224±92.98^{NS}	292±36.37^{NS}	305±100.70*	301.33±96.47*

The number between brackets indicates groups.
NS=Non-significant (P>0.05), * = Significant (P<0.05), ** = Highly significant (P<0.01), *** = Very highly significant (P<0.001).
WBCs count × 10^{-3}/mm^3, RBCs count × 10^{-6}/mm^3, HG (Hemoglobin) in g/dl, HCTs (Hematocrits) in %, No. of PLTs× 10^{-3}/mm^3

Table (2): Hematological changes in *Rattus albus* females as induced by *Vespa orientalis* stings

Groups and stings / Hematological parameters	Untreated groups (Mean ± SD)				Treated groups (Mean ± SD)			
	(1)	(2)	(3)	(4)	One sting (1)	Two stings (2)	Three stings (3)	Four stings (4)
WBCs	10.26± 4.55	11.23±3.78	13.46±1.79	10.70±3.70	8.63±1.41^{NS}	5.50±1.63*	6.46±1.62*	5.80±4.24*
RBCs	4.72±0.50	4.91±0.12	4.83±0.15	4.85±0.42	4.44±0.59^{NS}	4.10±0.40*	3.53±0.28*	3.25±0.75**
HG	12.46±1.26	15.50±3.83	13.23±1.27	12.96±0.15	11.60±1.80^{NS}	11.10±0.91*	9.50±0.17*	7.40±2.43**
HCTs	30.60±2.72	32.50±0.80	32.66±2.19	32.03±0.37	28.86±1.90^{NS}	28.43±2.27^{NS}	25±1.32**	19.76±7.19***
PLTs	165±68.82	205±57.29	155±60.91	173.33±50.85	163±50.56^{NS}	233±46.77^{NS}	171.33±79.82*	226±80.13*

The number between brackets indicates groups.
NS = Non-significant (P>0.05), * = Significant (P<0.05), ** = Highly significant (P<0.01), *** = Very highly significant (P<0.001).
WBCs count × 10^{-3}/mm^3, RBCs count × 10^{-6}/mm^3, HG (Hemoglobin) in g/dl, HCTs (Hematocrits) in %, No. of PLTs× 10^{-3}/mm^3

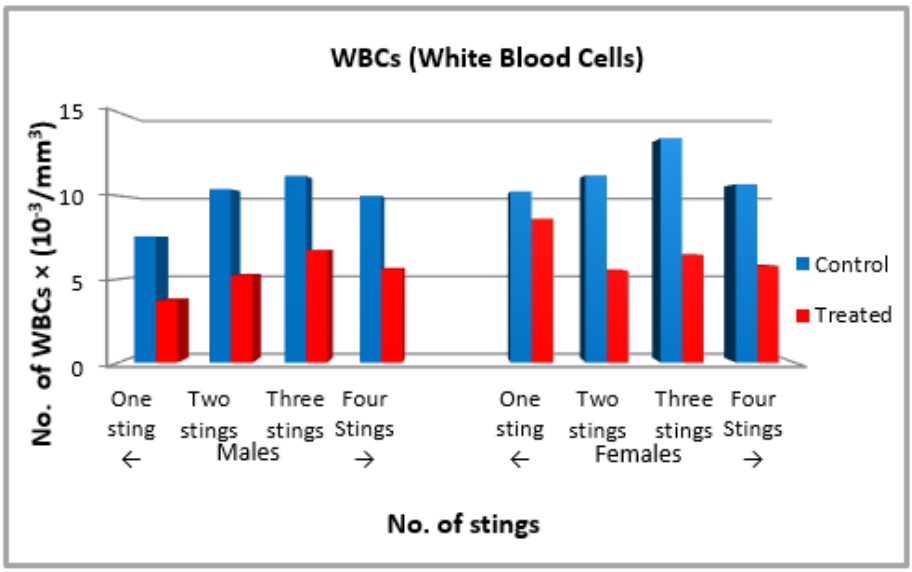

Fig. 1. Effect of *Vespa orientalis* stings on WBCs count in rat, *Rattus albus*

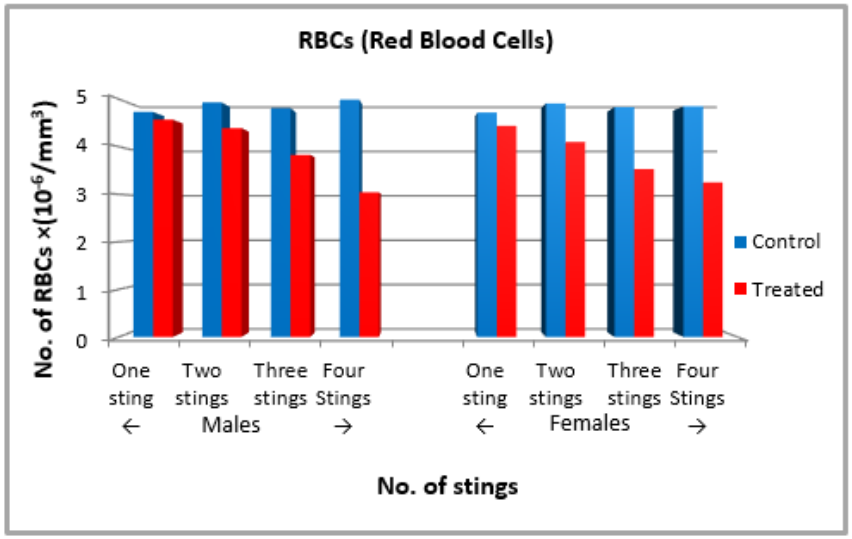

Fig. 2. Effect of *Vespa orientalis* stings on RBCs count in rat, *Rattus albus*

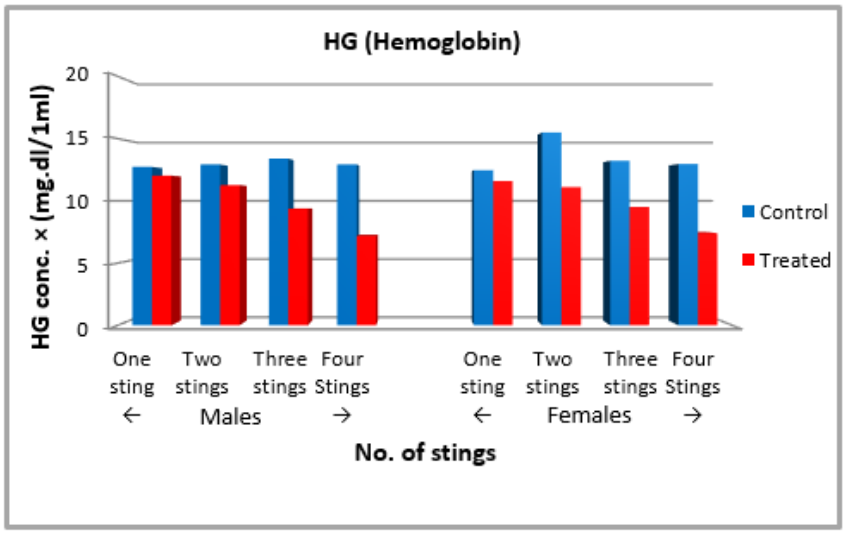

Fig. 3. Effect of *Vespa orientalis* stings on HG conc. in rat, *Rattus albus*

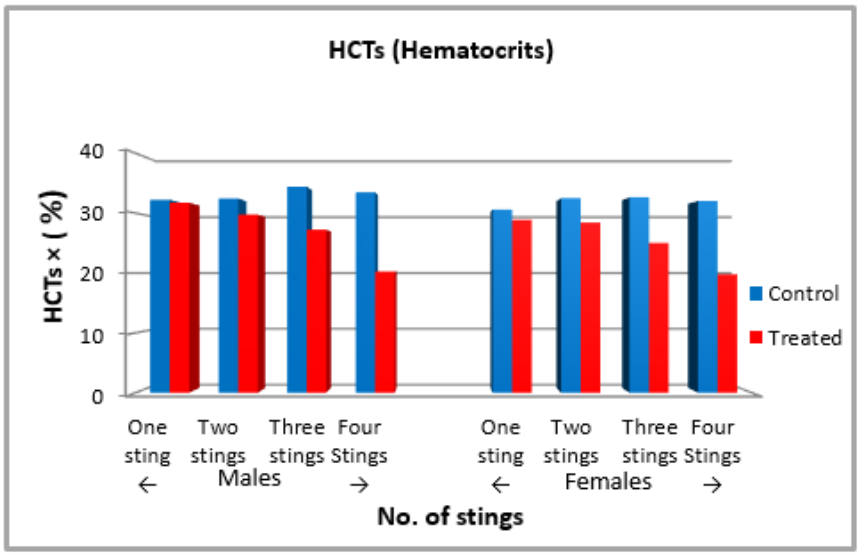

Fig. 4. Effect of *Vespa orientalis* stings on HCTs percentage in rat, *Rattus albus*

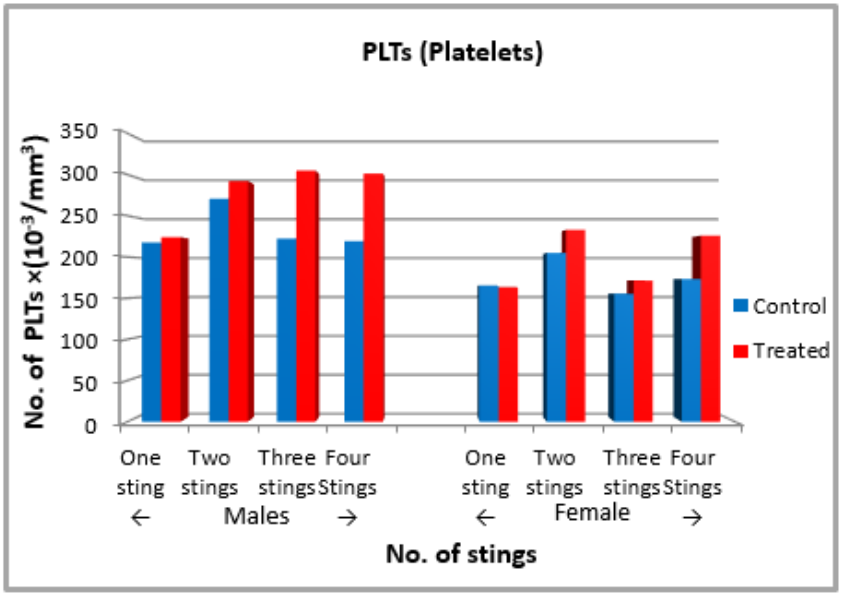

Fig. 5. Effect of *Vespa orientalis* stings on PLTs count in rat, *Rattus albus*

b. By Injection of Rats With Venom Sac Homogenate (VSH):

The results presented in tables (3&4) and figures (6-10) indicated the Hematological changes in rats (males & females) after injection with doses of 3mg/1ml saline solution, 6mg/1ml saline solution and 12mg/1ml saline solution from VSH of *Vespa orientalis*.

As shown from the results the two doses (6mg/1ml saline solution and 12mg/1ml saline solution) from VSH caused a significant decrease ($P<0.05$) and a highly significant decrease ($P<0.01$) in WBCs and RBCs counts, respectively in male rats.

Also, these two high doses from VSH caused a highly significant decrease ($P<0.01$) in the amount of HG of rat males. In addition, very highly decrease ($P<0.001$) in HCTs percentage was induced by these two doses. On the other hand, the aforementioned doses caused a significant increase in PLTs count in male rats.

In female rats, the three doses used (3, 6 and 12mg/1ml saline solution) caused significant decrease in erythrocyte count, where the mean count of the WBCs decreased from [10.73±2.55, 9.4±3.62 and 15.13±6.58 to 4.87±0.8, 2.33±3.26 and 6.03±1.10] × ($10^{-3}/mm^3$) by the doses of 3, 6 and 12mg/1ml saline solution, respectively.

The RBCs count significantly decreased from (5.11±0.14 to 4.65±0.29) × ($10^{-6}/mm^3$) by the dose of 3mg/1ml saline. However, the two high doses (6mg/1ml and 12mg/1ml) caused a very highly significant ($P<0.001$) decrease in the RBCs count.

Also, HG conc. was very highly significantly ($P<0.001$) decreased by these two high doses. In addition, a very highly significant decrease ($P<0.001$) in HCTs% was induced by these two high doses. On the other hand, all doses used caused a significant increase of PLTs count in female rats.

From the aforementioned results, it has appeared that the two high doses (6mg/1ml saline and 12mg/1ml saline) from the VSH of *V. orientalis* caused a significant decrease in WBCs, RBCs, HG and HCTs when injected to rats (males or females). While they caused a significant increase of PLTs when injected to male or female rats. However, the statistical analysis of data indicated a significant difference ($P<0.05$) between treated male and female rats in RBCs count, HG conc. and HCT %, where the decrease in these Hematological parameters was higher in female rats than male rats.

Table (3): Hematological changes in *Rattus albu*s males as induced by venom sac homogenate (VSH) injection

Groups and stings / Hematological parameters	Untreated groups (Mean ± SD)			Treated groups (Mean ± SD)		
	(1)	(2)	(3)	3mg/1ml (1)	6 mg/1ml (2)	12mg/1ml (3)
WBCs	9.10±3.29	14.73±4.40	12.83±1.18	6.80±3.03 ^{NS}	8.73±0.05*	5.26±2.40**
RBCs	5.38±0.04	5.13±0.17	5.33±0.11	5.15±0.04 ^{NS}	4.12±0.09***	3.85±0.38***
HG	13.73±0.57	14.33±0.77	14±0.86	13±0.43 ^{NS}	9.93±0.41***	9.46±1.10***
HCTs	33.53±0.15	32.26±1.09	33.30±0.70	31.66±0.416*	25.26±0.50***	22.80±2.45***
PLTs	214.66±17.38	176±9.16	233.66±48.88	215.66±27.53 ^{NS}	222.33±34.53*	240.66±60.27 *

The number between brackets indicates groups.
NS = Non-significant (P>0.05), * = Significant (P<0.05), ** = Highly significant (P<0.01), *** = Very highly significant (P<0.001).
WBCs count × $10^{-3}/mm^3$, RBCs count × $10^{-6}/mm^3$, HG (Hemoglobin) in g/dl, HCTs (Hematocrits) in %, No. of PLTs × $10^{-6}/mm^3$

Table (4): Hematological changes in *Rattus albu*s females as induced by venom sac homogenate (VSH) injection

Groups and Stings / Hematological parameters	Untreated groups (Mean ± SD)			Treated groups (Mean ± SD)		
	(1)	(2)	(3)	3mg/1ml (1)	6 mg/1ml (2)	12mg/1ml (3)
WBCs	10.73±2.55	9.4±3.62	15.13±6.58	4.87±0.8*	2.33±3.26*	6.03±1.10**
RBCs	5.11±0.14	5.24±0.15	5.40±0.08	4.65±0.29*	4.01±0.10***	3.71±0.24***
HG	13.10±0.52	13.10±0.69	14.13±0.15	12.13±0.72*	9.66±0.40***	8.86±1.02***
HCTs	31.60±0.52	32.53±1.60	33.70±1.40	29.60±1.30 ^{NS}	25.26±2.80***	22.46±0.65***
PLTs	201.66±14.57	228±30.80	200.33±85.58	224.66±18.23 *	284.33±36.115 **	220.66±35.44 **

The number between brackets indicates groups.
NS = Non-significant (P>0.05), * = Significant (P<0.05), ** = Highly significant (P<0.01), *** = Very highly significant (P<0.001).
WBCs count × $10^{-3}/mm^3$, RBCs count × $10^{-6}/mm^3$, HG (Hemoglobin) in g/dl, HCTs (Hematocrits) in %, No. of PLTs × $10^{-6}/mm^3$

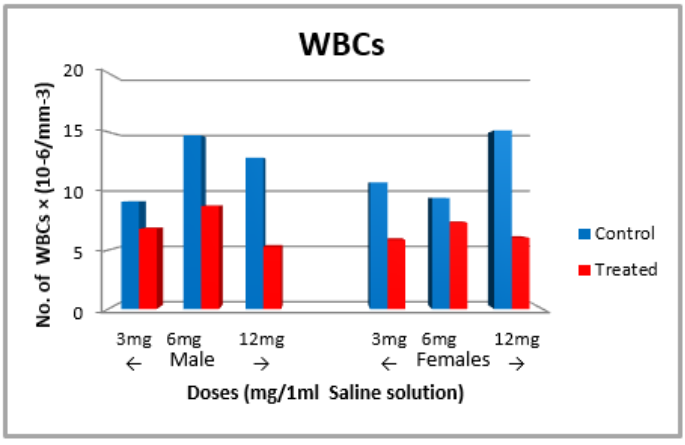

Fig. 6. Effect of *Vespa orientalis* VSH on WBCs count. in rat, *Rattus albus*

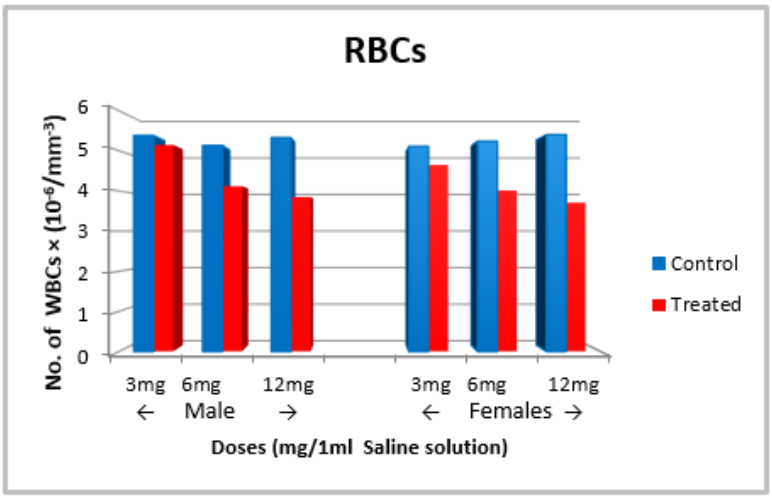

Fig. 7. Effect of *Vespa orientalis* VSH on RBCs count. in rat, *Rattus albus*

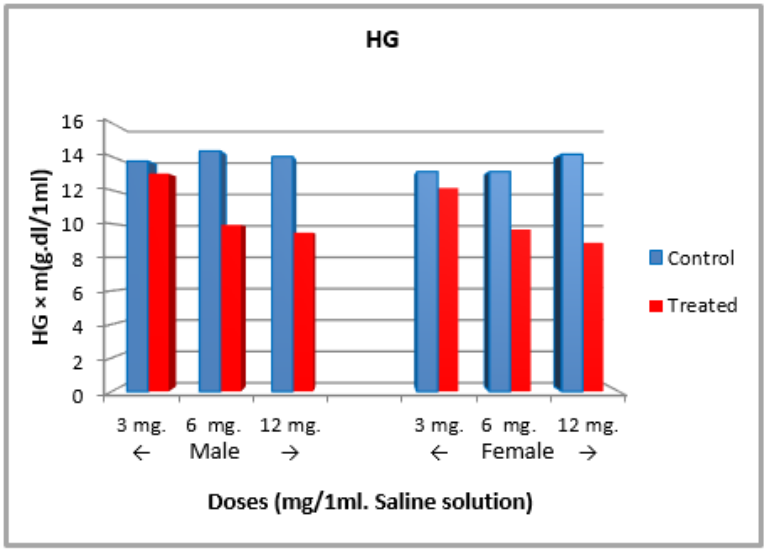

Fig. 8. Effect of *Vespa orientalis* VSH on HG conc. in rat, *Rattus albus*

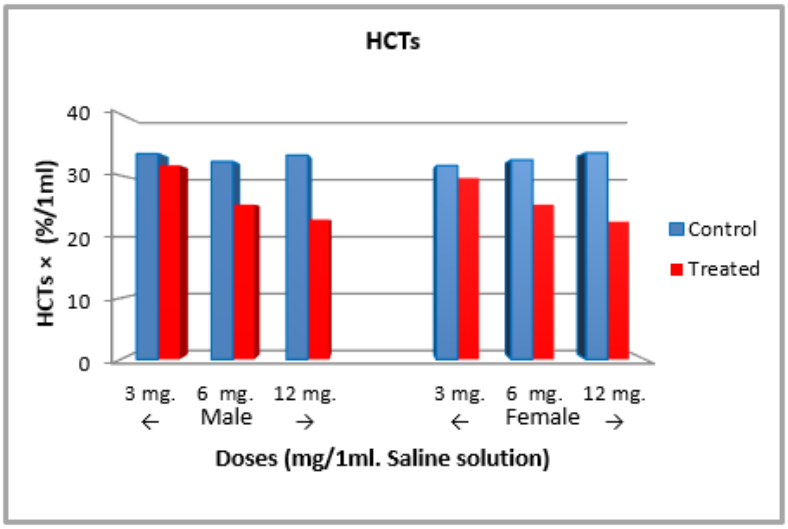

Fig. 9. Effect of *Vespa orientalis* VSH on HCTs percentage in rat, *Rattus albus*

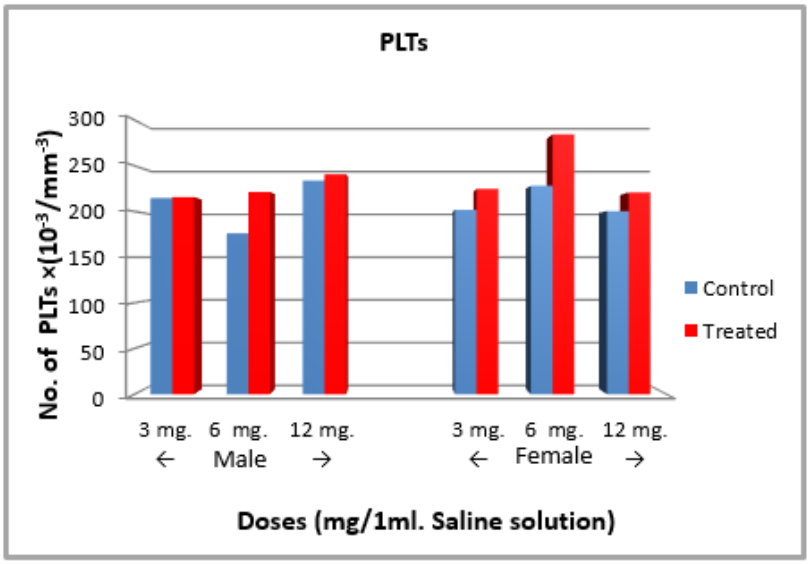

Fig. 10. Effect of *Vespa orientalis* VSH on PLTs count in rat, *Rattus albus*

DISCUSSION

Hematotoxic Effects:

The hemolytic activity of animal toxins has been extensively studied. Most of the studies concern the in vivo and in vitro hemolytic effects of snake and bee venom (Roy, 1945; Gitter et al., 1959; Klibansky and De Vries, 1963; Haberman, 1968). Wasp venoms have been relatively little studied, due to difficulties in obtaining their venom in sufficient amount. However, the presence of hemolytic factors in the venom of the oriental hornet (*V. orientalis*) has been reported (Joshua et al., 1971; Fischi et al., 1972 and Joshua and Ishay, 1973).

A large number of bioactive substances including toxic peptides, amines and proteins have been identified in wasp venoms. These venom components can act on the nervous, cardiovascular and immunological system of mammals (Mortari et al., 2012).

Vespa orientalis venom had been shown to contain several pharmacologically active substances including histamine, acetylcholine, 5-hydroxtryptamine, kinins, adrenaline, noradrenaline and dopamine (Edery et al., 1972).

In the present study, the stinging of rats by *V. orientalis* wasps or injection the rats with their venom sac homogenate (VSH) showed toxic effects on blood of rats, especially by three or four stings or by injection with high doses of VSH (6 and 12mg/1ml saline solution). These treatments evoked a significant decrease in WBCs, RBCs, HG conc., HCTs percent, but a significant increase of PLTs indicating that *V. orientalis* venom has a hemolytic activity on the in-vivo rat blood. These results are consistent with those obtained by Mortari et al., (2005) who reported that venom of *Polybia* wasp had a hemolytic activity or cytotoxic effect on washed human red blood cells.

Also, the present results are going in harmony with the results obtained by Joshua and Ishay (1973) using *V. orientalis* as they demonstrated that the venom hemolysed erythrocytes of man, guinea pig, rabbit, cat and rat in vitro.

The present study is the first to demonstrate cytotoxic effects on the blood of rat in vivo using the stings of *V. orientalis*.

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ARABIC SUMMARY

التأثيرات الهيماتولوجية السامة لسم الدبور الشرقي *Vespa orientalis* في الجرذ، *Rattus albus*

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تمت دراسة الخواص الدوائية لسم الدبور الشرقي؛ *Vespa orientalis* وسميته ضد الجرذان البيضاء؛ *Rattus albus* عن طريق اللسع المباشر من الدبور أو عن طريق الحقن بمطحون كيس سم الدبور. تم التحقق من درجة تحلل الدم والتغيرات الدموية الأخرى التي يسببها اللسع المباشر من الدبور أو الحقن بمطحون كيس سم الدبور داخل الجسم الحي للجرذ؛ *Rattus albus*. أظهرت النتائج التي تم الحصول عليها أن كلاً من اللسع المباشر من الدبور وحقن السم لهما تأثيرات سامة على دم الجرذان المختبرة، خاصة اللسعات المتعددة (3 أو 4 لسعات) والجرعات العالية من مطحون كيس السم VSH (6 و 12 ملجم / 1 مل محلول ملحي). أثارت هذه المعاملات انخفاضاً كبيراً في عدد كريات الدم البيضاء، عدد كريات الدم الحمراء، نسبة الهيموجلوبين ونسبة الهيماتوكريت، لكن حدثت زيادة ذات دلالة إحصائية (معنوية) في عدد الصفائح الدموية ممايشير إلى أن سم الدبور الشرقي *Vespa orientalis* له نشاط تحللي لمكونات الدم داخل الجسم الحي للجرذان.