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# WASP VENOM TOXIN INDUCED HEMATOLOGICAL CHANGES IN ALBINO MICE

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## ABSTRACT

In present investigation hematological effects of Yellow wasp *Polistes flavus* were determined in albino mice. Venom gland extract was purified on a Sepharose CL 6B 200 gel column at a constant flow rate in regular fractions. Pooled fractions were lyophilized and subsequently LD50 was determined. The $LD_{50}$ of the yellow wasp *Polistes flavus* venom protein was found 36.11 mg/kilogram body weight i.e., 0.03611 mg/gram body weight of albino mice. For visualizing hematological effects physiological or sub-lethal dose was administered in laboratory reared albino mice. The hemoglobin level in blood was significantly increased to maximum level i.e., 101.9%, 103.90%, 107.8% and 122.5% of the control at 2, 4, 6, 8, and 10 hour of treatment of 40% of 24-h $LD_{50}$ respectively. The number of red blood cells (RBCs) was decreased to 71.0% and 69.8% while numbers of the total white blood cells (WBCs) was increased to 136.3% and 132.6% of the control after 10 hours of treatment of 40% and 80% of 24-h $LD_{50}$ respectively. The level of mean corpuscular hemoglobin (MCH) was increased to 123.4% and 128.90% of the control at 8 hours of treatment with 40% and 80% of 24-h $LD_{50}$ of wasp venom. While the level of mean corpuscular hemoglobin decreased to 116.2% and 113.29% of the control after 10 hours of treatment with 40% and 80% of 24-h of $LD_{50}$ of wasp venom toxins. Low molecular weight toxic peptides ranging from 14.3-63 kDa were identified in wasp venom with strong cytotoxic action that caused intra-vascular hemolysis of RBCs, leucocytes, platelets and vascular endothelium. The sub-lethal venom doses (1/4 and 1/2 LD50) has induced unenviable increase in level of MCV, MCH, PCV, WBCs and show heavy RBC hemolysis and damage nerve cells.

**KEYWORDS:** *Polistes flavus*, venom toxins, RBCs, WBCs, PCV, MCV, MCH and plasma hemoglobin.

## INTRODUCTION

The poisonous animals are widely distributed throughout the world and their range starts from tiny unicellular protozoans to multi-cellular mammals but animals evolved defense organs such as fangs, nematocysts, spines, stings and pincers etc. Envenomation and poisoning by terrestrial animals mainly hymenopterans is a significant problem for health. These use venoms to make self-defense. Wasp venom toxins generate multiple organ dysfunction followed by anaphylactic reaction.[1] These impose multisystem changes and show wide range of biological activities such as intravascular hemolysis, rhabdomylysis, acute renal failure, cardiac, involvement, hepatic dysfunction and occasionally thrombocytopenia and coagulopathy. Honey bee toxins cause significant serological changes[2] and severely effect blood biochemical parameters and generate toxic effects in man.[3]

Functionally, the yellow wasp toxins are the highly active substances[4], which blocks the ionic channels of the cell and causes fast death.[5] In sufficient concentration cytotoxins causes cytolysis, a process in which soluble molecules are leaking out from the cells, but in low concentration they may cause mild damage to the plasma membrane.[6] Yellow wasp envenomation in large numbers are fatal to human as well as animals and it causes severe inflammation, swelling, rhabdomylysis, renal-insufficiency, cardiac-involvement, hepatic dysfunction and occasionally thrombocytopenia and coagulopathy and severe pain.[7] Yellow wasp *Polistes flavus* venom causes heavy hemolysis and does make significant alteration in other hematological parameters.[8] Different toxins with diverse functional and structural composition have been reported from so many animals and plant species.[9] Among them animal venoms particularly insect's venom are potential toxins, which are used for either for predation or in social defense.

Wasp venom shows antimicrobial, insecticidal and hemolytic properties.[10] The crude venom of *Paulibia paulista* venom shows significant levels of hyaluronidase, phospholipase, and protolytic, hemolytic, and myotoxic reactions or activities.[11] Hornatins are the basic proteins which is isolated from *Vespa flavitarsus* shows hemolytic activities in the red blood cells and pre-synaptic nerve cells.[12] These also possess catalytic activities to hydrolyze emulsified phosphatidylcholine but do not act up on sphingomyelin. Melitins are also interacts with the

RBC membrane and induce biochemical changes and disorders in lipids proteins matrix both in hydrophobic core of the lipid bilayer and at polar/non-polar interface of RBC membranes.[8] Hymenoptera venom contains proteins, polypeptides and low molecular weight aromatic and aliphatic constituents in variable amounts. It also contains some important enzymes i.e., phospholipase A, hyaluronidase, acid phosphatase and D-glucosidase that are highly antigenic. The hyaluronidase acts as a spreading factor that allows the toxic substances to infiltrate the tissues and rupture the blood cells. Envenomation in-group are highly fatal to humans as it causes severe inflammation, swelling, rhabdomylysis, renal-insufficiency and severe pain. After few seconds of envenomation, toxins cause heavy RBC hemolysis and damage nerve cells[13] and biochemical functions of enzymes and proteins are also inhibited. Wasp toxins cause allergy by immune stimulation of the body.

Melittin is also known as "surface active peptide". It is tetrameric and possesses ionophore properties.[14] Melittin is strongly antigenic and causes tissue irritation. It causes a constant skin nerve terminal depolarization and intense pain after stinging.[15] Phospholipase-A is an enzyme that hydrolyses It possess 26 amino acid residues with amphypathic characteristics (polar and non-polar ends), which allows it to interact with lipid membranes, which in turn, can increase permeability of the erythrocytes and other cell membranes.[16][4][17-19] The esters bonds of phospholipids at the sn-1 position and produce 2-acyl-lysophospholipids and fatty acids. Vespid venom phospholipase-A belongs to the pancreatic lipase family and exhibits membrane binding activities. $PLA_2$ disturb the phospholipids packing from the several types of the biological membrane leading to pore formation and due to its effects cell lysis process become start.[20,21]

In experimental animals venom administration severally effects blood biochemical parameters and generates toxic effects.[22] The most common type of reaction to an insect sting is a local reaction to the bite of a mosquito (a small fly of the family Culicidae). The reaction reflects an allergic response to proteins in the insect saliva leading in about ¾ of all to an immediate allergic reaction (wheal) and about ½ to a delayed reaction (papule). Hymenopteran insects induce IgE-mediated systemic allergic reactions which are of greater clinical significance. They show an immediate (anaphylactic) response that can have fatal consequences. Certain species of wasp belonging to family Vespidae example *Vespula vulgaris* and *Vespula germanica* cause anaphylaxis that is occasionally caused by others species of Vespidae such as *Dolicho vespula* species, hornet *Vespa crabro* and bees (mainly

bumblebees).[23]. *Vespa tropica* (VT) venom shows anti-inflammatory potentials of venom.[24] Mast cells (MC) are effector cells which display severe systemic reactions (SR) to Hymenoptera stings.[25-27] In the present research investigation wasp venom induced hematological alterations such as RBCs, WBCs, PCV, MCV, MCH and plasma hemoglobin in albino mice were established.

## MATERIALS AND METHODS

### 1. Collection of yellow wasp

The living yellow wasp *Polistes flavus* were collected from different regions of Gorakhpur city. The collected wasps were immobilized by quick freezing at -20° C. The venom glands were taken out by cutting the last two segment of abdominal region of wasp and these were homogenized in phosphate buffer saline (50 mM, pH 6.9) with the help of power homogenizer. The homogenate was centrifuged at 10000 rpm at 4°C for 10 minutes and the supernatant was used as crude venom.

### Molecular weight determination of purified venom proteins

Range of molecular weight of different proteins/toxins in the purified wasp venom was determined by running the proteins of known molecular weight through Sepharose CL-6B gel column as done previously at the same flow rate. A calibration curve was drawn between Ve/Vo log M and with the help of calibration curve range of molecular weight of different protein in the purified yellow wasp *Polistes flavus* venom was determined.

### Lyophilization of eluted venom protein

The eluted fractions containing venom proteins were pooled and lyophilized to a desired concentration of venom toxins proteins.

### Biological activity of the Purified Yellow wasp *Polistes flavus* venom protein

Biological activity testing of *Polistes flavus* toxins was determined in the Cockroach (both adult and nymphs) and albino mice. For this purpose very low volume (14 µl) of purified toxins in solution were injected in the thoracic region of insects. In albino mice serially known volumes of the *Polistes flavus* venom toxins were injected intra-peritoneal. For myocardial activity, small amount of purified venom toxin was injected neck region of mice. For cytotoxic assays, erythrocytes of rat and man were suspended in buffer and toxins were directly mixed in the buffer. The number of visible cells was counted by using Trypan blue dye.

**4 (A) Determination of the lethality of *Polistes flavus* venom toxins**

The albino mice were injected subcutaneously with the purified venom toxins of different serial concentration and $LD_{50}$ was determined at the intervals of the 24 hours. Deformities such as paralysis and neurotoxic effects were also recorded. Mortality was determined by using Abbot's formula. The $LD_{50}$ values were calculated at which half of the test animals were died. The lethal concentration for 40% and 80% of the $LD_{50}$ was determined with the doses-mortality regression line plotted on the log Probit method's.[28] The confidence limits were calculated at 95% probability levels.

**5(A) Determination of the hematological parameters (*in vivo)***

**1. Determination of blood hemoglobin**

Blood hemoglobin was measured by the method of.[29] The blood was sucking in the pipette up to 0.2 marks. It was transferred in the clean hemoglobinometer tube containing small amount of N/10 HCl. The contents were allowed and shake well 10 minutes until the blood was converted into hematic acid. Now the N/10 HCl was added drop by drop into the tube and stirred continually with a glass rod. This was continued until the color of the contents exactly matched with the color of adjacent standard tubes. The end point was noted from where the color was matched to the standard tube which shows the hemoglobin percentage.

**2. Determination of packed cell volume (PCV)**

In this process 1 ml blood was collected from the mice by adding EDTA as an anticoagulant. It was centrifuged at 10000 rpm for 20 minutes for the separation of plasma and blood cells. After separation, dark color settled blood cells and yellowish plasma was obtained. Packed Cell Volume (PCV) will calculated by the following formula and it was expressed in % PCV**.**

PCV= volume of blood corpuscles ×100/volume of blood

**3. WBCs counting**

The blood was sucked up to the 0.5 marks and immediately diluted the blood with PBS buffer (pH 6.9) up to 11 marks and allowed it to mix WBC diluting fluids. Put a drop of blood solution on a clean counting hemocytometer and covered with the help of cover slip. The corpuscles was allowed to settle down and counted under the microscope. The counting was performed in the four corners of 1 $mm^{3.}$ The number of WBCs per $mm^3$ of the blood was calculated by the formula- WBCs number= No. of WBCs counted× dilution ×20/No. of squares counted $mm^3$.

**4. RBCs counting**

From each mice blood was taken out and sucked in pipette up to 0.5 ml marks by avoiding any air bubbles. Immediately, Hayem's diluting fluids was sucked in the pipettes up to 10 marks and then mixed. Few drops of RBCs solution was put on the hemocytometre and covered with cover slip and allowed to settle down for 1 minute. The RBCs were counted under the microscope in 5 groups each containing 16 small squares. Thus the counting was done in 80 small squares. The total numbers of the RBCs will calculated by the formula-

RBCs number= No. of RBCs counted× dilution×4000/No. of small squares.

**5. Determination of plasma hemoglobin**

For this 0.2 ml of the blood was taken by the puncher of the cardiac muscles and quickly poured it into centrifuge tube already having EDTA. Now the blood was centrifuged at 10000 rpm for 5 minutes and the upper yellowish plasma layer was taken out for the measurement of hemoglobin. Furture process was same as the blood hemoglobin.

**6. Determination of mean corpuscular hemoglobin (MCH)**

It was calculated by the amount of hemoglobin per liter (in gram) by the Red Blood Cells in million/$mm^3$.

**5(B) *in vitro* study of hemolytic activity**

*In vitro* hemolysis assays, bloods were incubated with different concentration of purified *Polistes flavus* wasp venom 5µg, 10µg, 15µg, 20µg and 25µg. Further process was followed similar to as *in vivo* experiments.

**Ethical Issues**

The experiments were performed by following the guideline of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India and experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).

## RESULT

### Molecular weight determination of wasp venom toxins

Molecular weight of *Polistes flavus* venom toxins/proteins was determined by Sepharose CL-6B 200 gel column chromatography using standard marker proteins of known molecular weight (Figure 1). The calibration curve indicates that the molecular weight of purified venom proteins ranging from 14.3-63 kDa (Figure 2).

### Venom toxicity

The eluted fractions of venom proteins were pooled and lyophilized. The toxicity of the purified wasp venom toxins of the *Polistes flavus* toxin was determined against albino mice (*Mus musculus*). The yellow wasp venom proteins obtained from the lyophilization of the two peaks caused toxicity in the albino mice. The $LD_{50}$ of the yellow wasp *Polistes flavus* venom protein was found 36.11 mg/kilogram body weight i.e., 0.03611 mg/gram body weight of albino mice.

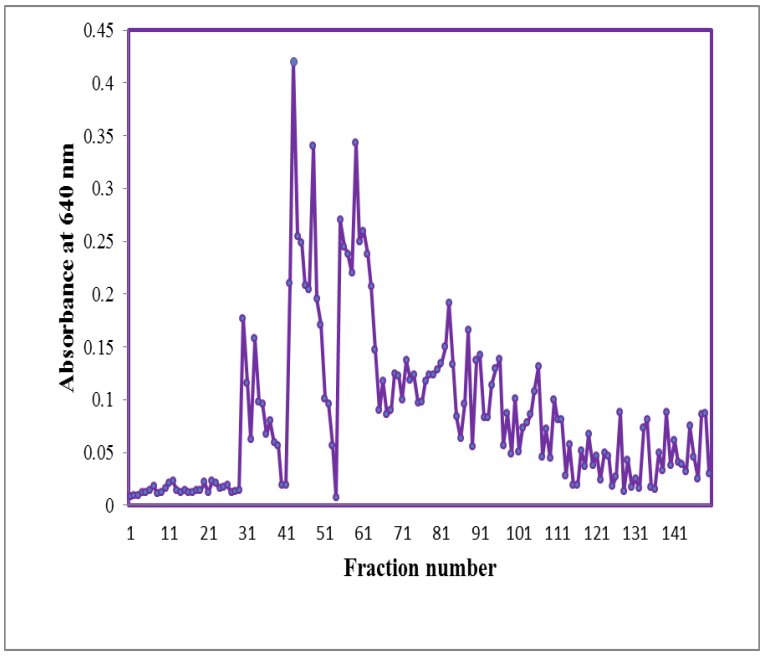


**Figure 1: Elution pattern of phosphate buffer (50 mM, pH 6.9) extractable venom proteins of yellow wasp *Polistes flavus* chromatographed on Sepharose CL-6B column. Absorbance was taken at 640 nm.**

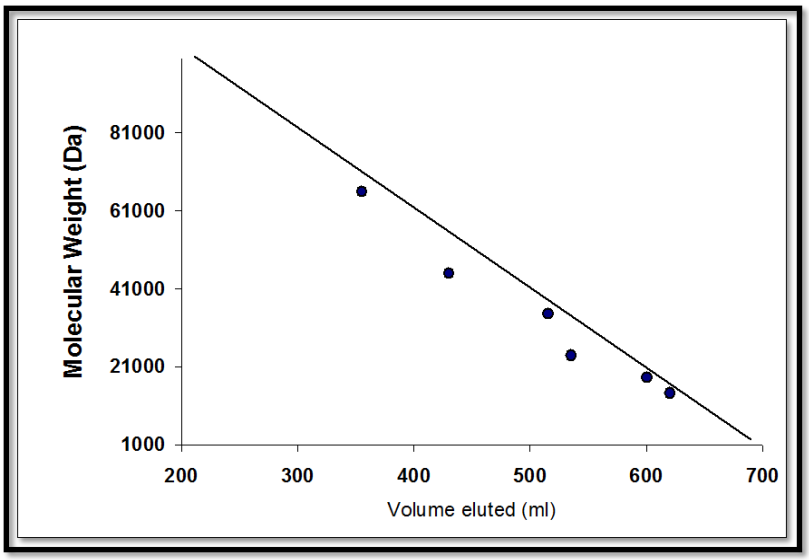


**Figure 2: Standard proteins chromatographed on Sepharose CL-6B 200 column for determining the molecular weights of venom proteins/peptides isolated from *Polistes flavus*. Proteins used were bovine albumin mol. wt 66,000, egg albumin mol. wt. 45,000, pepsin mol. wt. 34,700, trypsinogen mol. wt. 24,000, beta lactoglobulin mol. Wt18,400 and lysozyme mol. wt. 14, 300. Elution volumes of unknown proteins were compared with log values on the X-axis for estimation of molecular weights.**

**SECTION (II)**

**(I). *In vivo* effects of purified venom toxins from yellow wasp *Polistes flavus* on hematological alterations in Albino mice**

This section deals with the study of the effects of the purified venom toxins from *Polistes flavus* on blood hemoglobin, packed cell volume (PCV), plasma hemoglobin, red blood cells, total white blood cells and mean corpuscular hemoglobin (MCH) in albino mice.

Mice were treated with 40% and 80% of 24-h $LD_{50}$ of purified venom toxins of *Polistes flavus* and different parameters were measured after 2, 4, 6, 8 and 10 hours of treatment. Yellow wasp *Polistes flavus* venom caused significant ($p<0.05$) reduction in red blood cells and increase in blood hemoglobin, mean corpuscular hemoglobin, packed cell volume, total white blood cells and plasma hemoglobin (Table 1 & 2).

The hemoglobin level in blood was significantly increased to maximum level i.e., 101.9%, 103.90%, 107.8% and 122.5% of the control at 2, 4, 6, 8, and 10 hour of treatment of 40% of 24-h $LD_{50}$ respectively. The hemoglobin level was increased to maximum 115.6%, 121.5%,

and 129.4% of the control at 6, 8, and 10 hour of treatment at 80% of 24-h $LD_{50}$ respectively (Table 1 & 2; Figure 3).

The maximum increase in the packed cell volume (PCV) was obtained 2.5–2.55 times at 10 hour of treatment of 40% and 80% of 24-h $LD_{50}$ (Figure 4).

Plasma hemoglobin level was increased 111.6% and 115.0% of the control after 10 hours of treatment of 40% and 80% of 24-h $LD_{50}$ respectively (Table 1 & 2; Figure 5).

The number of red blood cells (RBCs) was decreased to 71.0% and 69.8% of the control after 10 hour treatment with 40% and 80% of 24-h $LD_{50}$ of the wasp venom toxins (Table 1& 2; Figure 6).

The numbers of the total white blood cells (WBCs) was increased to 136.3% and 132.6% of the control after 10 hours of treatment of 40% and 80% of 24-h $LD_{50}$ respectively (Table 1 & 2; Figure 7).

The level of mean corpuscular hemoglobin (MCH) was increased to 123.4% and 128.90% of the control at 8 hours of treatment with 40% and 80% of 24-h $LD_{50}$ of wasp venom. While the level of mean corpuscular hemoglobin decreased to 116.2% and 113.29% of the control after 10 hours of treatment with 40% and 80% of 24-h of $LD_{50}$ of wasp venom toxins (Table 1 & 2; Figure 8).

The variation in red blood cells, hemoglobin, MCH, total white blood cells, packed cell volume and plasma hemoglobin was time dependent and dose dependent ($p<0.05$, f-test, student t-test).

**Table 1: *In vivo* effects of 40% of 24-h $LD_{50}$ of purified venom toxins from *Polistes flavus* on the Blood hemoglobin, PCV, plasma hemoglobin, number of RBCs, WBCs, MCH in albino mice.**

| Parameters | Time in hours | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Hemoglobin | 10.2±0.081 (100.0) | 10.3±0.081 (100.9) | 10.4±0.081 (101.9) | 10.6±0.081 (103.9) | 11.2±0.081 (107.8) | 12.5±0.081 (122.5) |
| PCV | 20.0±0.047 (100.0) | 25.0±0.047 (135.0) | 27.5±0.047 (137.5) | 40.0±0.047 (200.0) | 45.0±0.047 (225.0) | 50.0±0.046 (250.0) |
| Plasma Hb | 0.06±0.081 (100.0) | 0.061±0.081 (102.2) | 0.062±0.081 (104.3) | 0.063±0.081 (105.8) | 0.065±0.081 (108.7) | 0.067±0.08 (111.6) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **RBCs** | 3.45±0.008 (100.0) | 3.30±0.007 (95.6) | 3.06±0.007 (88.6) | 2.87±0.007 (83.1) | 2.69±0.006 (77.9) | 2.45±0.007 (71.0) |
| **WBCs** | 2.94±0.061 (100.0) | 3.09±0.061 (105.1) | 3.28±0.060 (111.5) | 3.51±0.07 (119.3) | 3.79±0.08 (128.9) | 4.01±0.069 (136.3) |
| **MCH** | 7.52±0.008 (100.0) | 7.70±0.008 (102.3) | 8.10±0.008 (107.7) | 8.62±0.008 (114.6) | 9.28±0.008 (123.4) | 8.74±0.008 (116.2) |

Values are mean ± SE of three replicates

Values in parentheses indicates percentage level with control taken as 100%

*Significant ($p<0.05$, Student t-test)

RBCs: Red Blood Cells, and Hb: hemoglobin

WBCs: White Blood Cells

MCH: Mean Corpuscular Hemoglobin

PCV: Packed Cell Volume

**Table 2: *In vivo* effects of 80% of 24-h $LD_{50}$ of purified venom toxins from *Polistes flavus* on the Blood hemoglobin, PCV, plasma hemoglobin, number of RBCs, WBCs, MCH in the albino mice.**

| **Parameters** | **Time in hours** | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **2** | **4** | **6** | **8** | **10** |
| **Hemoglobin** | 10.2±0.081 (100.0) | 12.2±0.081 (119.6) | 11.4±0.081 (111.7) | 11.8±0.081 (115.6) | 12.4±0.081 (121.5) | 13.2±0.081 (129.4) |
| **PCV** | 20.0±0.047 (100.0) | 30.0±0.047 (150.0) | 39.0±0.047 (195.0) | 43.0±0.047 (215.0) | 47.0±0.047 (235.0) | 55.0±0.046 (255.0) |
| **Plasma Hb** | 0.06±0.081 (100.0) | 0.062±0.081 (103.4) | 0.063±0.081 (105.7) | 0.064±0.081 (108.2) | 0.067±0.081 (112.6) | 0.069±0.081 (115.0) |
| **RBCs** | 3.45±0.007 (100.0) | 3.10±0.007 (89.8) | 3.00±0.007 (86.9) | 2.81±0.007 (81.4) | 2.52±0.007 (73.0) | 2.41±0.007 (69.8) |
| **WBCs** | 2.94±0.061 (100.0) | 3.10±0.061 (105.4) | 3.34±0.060 (113.6) | 3.60±0.07 (122.4) | 3.85±0.08 (130.9) | 3.90±0.069 (132.6) |
| **MCH** | 7.52±0.008 (100.0) | 7.90±0.008 (105.0) | 8.90±0.008 (118.3) | 9.09±0.008 (120.8) | 9.70±0.008 (128.9) | 8.52±0.008 (113.29) |

Values are mean ± SE of three replicates

Values in parentheses indicates percentage level with control taken as 100%

*Significant ($p<0.05$, Student t-test)

RBCs: Red Blood Cells and Hb: Hemoglobin

WBCs: White Blood Cells

MCH: Mean Corpuscular Hemoglobin

PCV: Packed Cell Volume

**(II). *In vitro* effects of the purified yellow wasp venom on red blood cells**

For determination of *in vitro* effects of wasp venom toxins, five different concentrations of purified yellow wasp *Polistes flavus* venom toxins i.e., 70µg, 140µg, 210µg, 280µg and 350µg were used for hemolytic assays. *In vitro* incubation of RBCs with purified wasp toxin caused dose dependent ($p<0.05$, f-test, student t-test) lysis of red blood cells. The percent hemolysis *in vitro* was found 12.3%, 19.9%, 30.3%, 47.8% and 67.7% at 70µg, 140µg, 210µg, 280µg and 350µg pre-incubation of purified yellow wasp *Polistes flavus* venom respectively (Table 3; Figure 9).

**Table 3: *In vitro* effects of different concentrations of purified venom toxins from *Polistes flavus* on red blood cells of albino mice.**

| S.N. | Doses in µg | % Hemolysis |
|---|---|---|
| 1. | 0 µg | 0.00±0.001 |
| 2. | 70 µg | 12.3±0.008 |
| 3. | 140 µg | 19.9±0.008 |
| 4. | 210 µg | 30.3±0.008 |
| 5. | 280 µg | 47.8±0.0081 |
| 6. | 350 µg | 67.7±0.0082 |

Values are mean ± SE of three replicates

Values in parentheses indicates percentage level with control taken as 100%

*Significant ($p<0.05$, Student t-test)

RBCs: Red Blood Cells

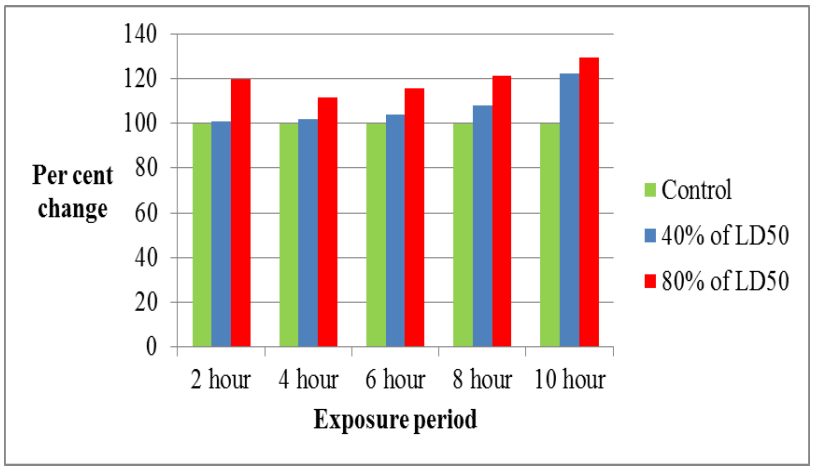


**Figure 3: *In vivo* effect of 40% and 80% of 24-h $LD_{50}$ of purified venom toxins of yellow wasp *Polistes flavus* on hemoglobin% of albino mice.**

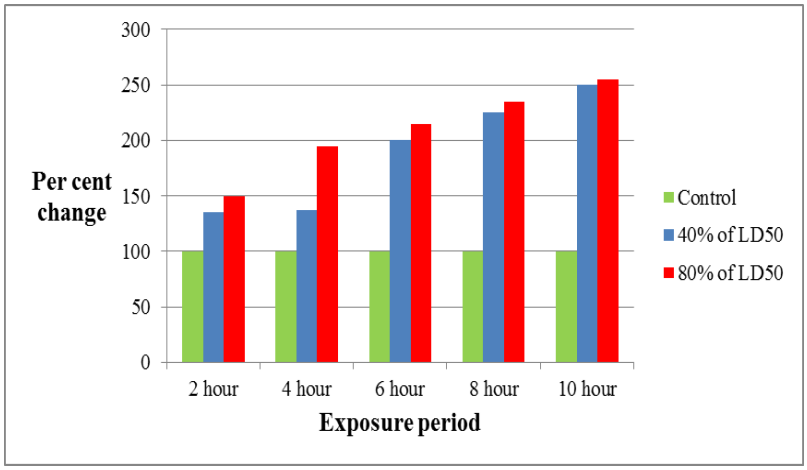


**Figure 4: *In vivo* effect of 40% and 80% of 24-h $LD_{50}$ of purified venom toxins of yellow wasp *Polistes flavus* on PCV of albino mice.**

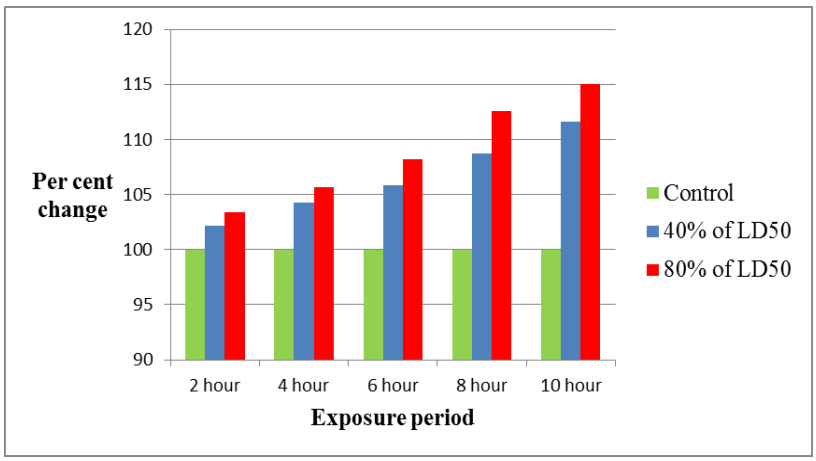


**Figure 5: *In vivo* effect of 40% and 80% of 24-h $LD_{50}$ of purified venom of yellow wasp *Polistes flavus* on Plasma hemoglobin of albino mice.**

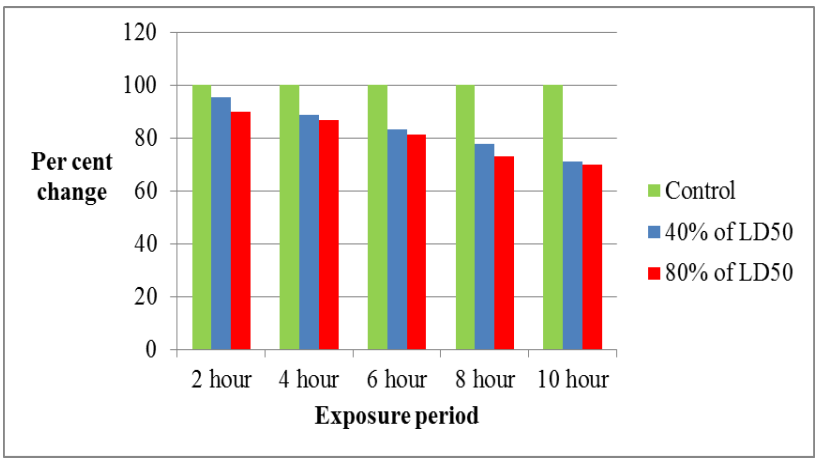


**Figure 6: *In vivo* effect of 40% and 80% of 24-h $LD_{50}$ of purified venom toxins of yellow wasp *Polistes flavus* on RBCs of albino mice.**

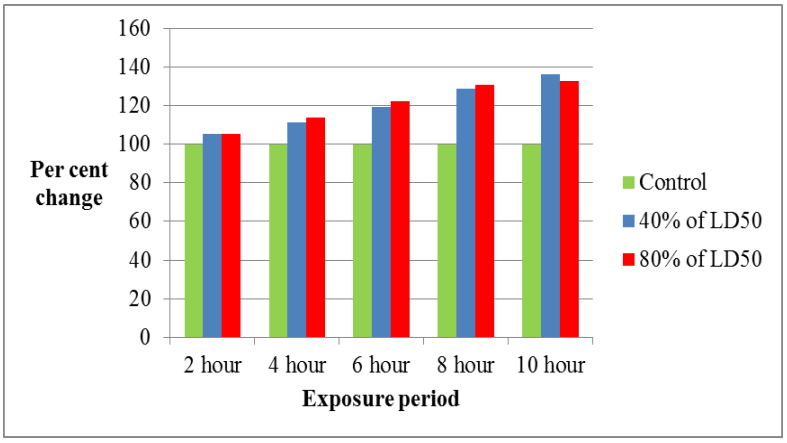


**Figure 7: *In vivo* effect of 40% and 80% of 24-h $LD_{50}$ of purified venom toxins of yellow wasp *Polistes flavus* on WBCs of albino mice**

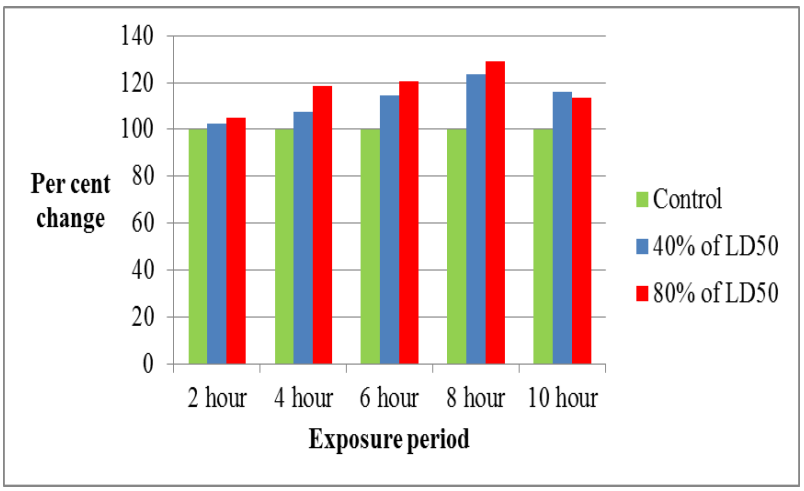


**Figure 8: *In vivo* effect of 40% and 80% of 24-h $LD_{50}$ of purified venom toxins of yellow wasp *Polistes flavus* on MCH of albino mice.**

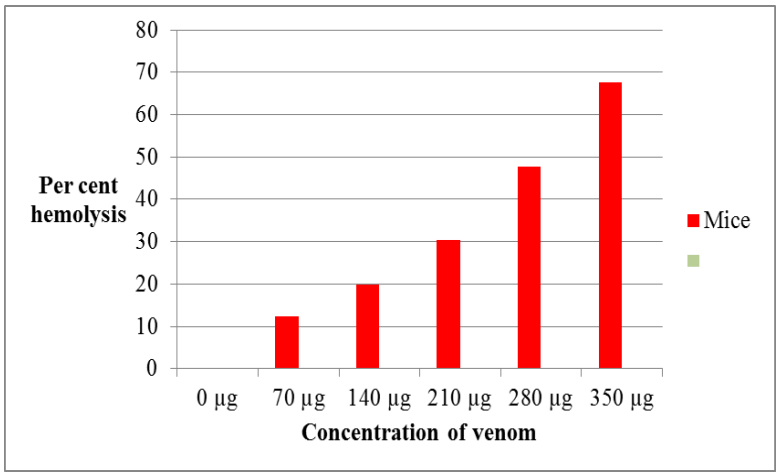


**Figure 9: *In vitro* effect of different concentration of purified yellow wasp *Polistes flavus* venom on red blood cells (RBCs) of albino mice.**

## DISCUSSION

Every year millions of wasp envenomation cases are reported from Southeast Asian countries, including India, Vietnam, Thailand, Malaysia, and Nepal. Wasp stinging is a major health problem for human beings in rural and urban areas. After feeling little disturbance in territory or in the close vicinity of colony hive, wasps suddenly attack on the passerby, free dwellers, children and inflict venom by the means of stinger.[30,31] Wasps swarms in group and forage individually in near surroundings of their hive. Accidently or incidentally they attack passerby, free dwellers, farmers, wagers and children.

The wasp venom is highly toxic to small mammals as it imposes sever tissue irritation, swelling and inflammation and pain in muscles. It caused early and delayed hypersensitivity, inflammatory reaction, necrosis and toxic complications in mammals and even invertebrates animals.[32] In present study number of red blood cells (RBCs) was decreased up to 71.0% and 69.8% of the control after 10 hour treatment with 40% and 80% of 24-h $LD_{50}$ of the wasp venom toxins.[33] More specifically wasp venom toxins also damaged nerve cells[33] and show intense myotoxic reaction to humans.[34] It leads to consequent loss of intracellular potassium and accumulation of sodium within the cytoplasm, oxidative damage and high lipid peroxidation.[35] From damaged RBCs and due to loss of parts of the membrane more hemoglobin (Hb) comes out in the serum.[36] The maximum increase in the packed cell volume (PCV) was obtained 2.5–2.55 times at 10 hour of treatment of 40% and 80% of 24-h $LD_{50}$ (Figure 6).

Wasp venom contains melittin a toxic peptides with alkaline nature, which is responsible for intense local pain[37], inflammation[18], itching, swelling, redness and irritation as immediate responses.[38] Melittin exhibits amphypathic properties (polar and non-polar ends), which allows it to interact with lipid membranes, which in turn, can increase permeability of the erythrocytes and other cell membranes[4] Melittin displays cytotoxicity and cause heavy hemolysis of RBCs[6] Melittin puncture the plasma membrane.[18] However, *in vitro* study it was come out those RBCs was the main target of wasp venom toxins.[39] Melittin induces the disorders in lipid-protein matrix both in the hydrophobic core of bilayer and at the polar/non-polar interface of melittin complexed with erythrocyte membranes.[40] Melittin effectively immobilizes membrane proteins in the plane of the lipid bilayer. Interaction of melittin with erythrocyte membranes generate local conformational changes in membranes, that is the main

reason of rupturing of red blood cells.[4][40] More specifically, melittin is cytotoxic while apamin shows neurotoxic effects.

The tetrameric form of the wasp venom shows ionophore properties[14], which is responsible for the constant skin nerve terminal depolarization that causes severe pain at the sting site.[41] Wasp venom also triggers cell lysis in many cell types and intercellular membranes, such as lysosomes.[42] It results in severe inflammation, swelling, rhabdomylysis, renal-insufficiency and severe pain.[7] Wasp sting also causes mild local reaction like weal formation around the sting sites with surrounding erythema to severe systemic reactions. Other than the above local reactions, wasp venom imposed some major damages such as intravascular hemolysis, acute renal failure, pulmonary and cerebral edema and pancreatitis to the patient's body.[43,44] Wasp venom poisoning causes many physiological alterations in man and increases the risk of death. Such accidents occur due to intra-dermal mass envenomation in a large number that produced hyperthermic effects in man.[45]

Wasp venom contain melittin, phospholipase A2 (PLA2), hyaluronidases bioactive substances which directly interact with cell membrane disrupt its integrity and generate immune allergic responses. But, most of the serious reactions reported in victims stung by wasps are allergenic in nature. However, 50-80% of victim's were reported to synthesize IgE-specific molecules to venom compound.[38] Some active substances of wasp venom toxin block the ionic channels and damage nervous system.[5] Melittin and phospholipase A2 play an important role in induction of allergic reactions and causes irritation in patients. Few other components like histamine and nor epinephrine cause hypertension, flushing and dysrhydumea.[46] Initial hypertension was observed after from the 30-40 minutes of venom injection in albino mice. This was due to action of neurotoxic peptide present in the *Polistes flavus* venom, which possibly inhibits the potassium channel and caused hypersensitive response.[47] But, after 50 minute of venom injection experimental mice has displayed hypotension, which is a serious problem after envenomation.[48] Wasp venom is strongly antigenic and highly toxic to man and possesses pharmacological properties.[49]

**Interaction with membranes**

Yellow wasp *Polistes flavus* venom shows cytotoxic activities with hematological immune alterations. It is potentially harmful to humans because it causes heavy RBCs destruction and show heavy release hemoglobin and carbonic anhydrase.[19] It stimulates synthesis of hemolytic substances, after 30 minutes. Rate of hemolysis become very high with the time, in

such a condition extra-corpuscular hemoglobin cannot convert into bilirubin as it quickly as it released. For the venoms, hemolytic activity correlated with neither lethal toxicity ($LD_{50}$) nor algogenicity. Therefore, changes in hemoglobinuria are increased. When plasma hemoglobin concentration exceeds the hemoglobin binding capacity of kidney tubular cell get decreased. Therefore, excess free plasma hemoglobin is filtered and excreted in the urine. The excess of both hemoglobin leads to acute tubular necrosis and renal failure. It may be due to direct wasp venom toxicity.[50] In wasp venom injected mice due to hepatic injury amino-transferase level become high which was improved subsequently.

Wasp venom phospholipase A2 is a membrane-bound phospholipid containing enzyme that is important in the production of arachidonic acid. Besides this, it also contains many low molecular weight compounds such as serotonin, histamine, acetylcholine, hyaluronidase and several kinins.[51] Yellow wasp *Polistes flavus* venom toxins have shown very high toxicity which is proved by very low $LD_{50}$ i.e. 0.03611 mg/gm of body weight obtained in albino mice.

Besides this, in the present investigation wasp venom toxins have shown significant ($p<0.05$) changes in certain hematological parameters such as blood hemoglobin, packed cell volume, white blood cells, red blood cells, plasma hemoglobin, mean corpuscular hemoglobin. After injection of sub-lethal dose of wasp venom a very heavy cell lysis was observed due to which blood hemoglobin concentration was increased after *Polistes flavus* venom injection in albino mice. Similar results were reported by[8] on toad blood and found significant increase in white blood cells, blood hemoglobin, plasma hemoglobin packed cell volume and mean corpuscular hemoglobin, while a transient decrease in red blood cells.

In the present study purified *Polistes flavus* venom toxins caused a significant ($p<0.05$) decrease in RBC count (69.8%) (Table 3 & Figure 8) and a frequent increased in number of white blood cells (136.3%) in comparison to control (Table 2 & Figure 9). Wasp envenomation caused decreased in the numbers of erythrocytes (69.8% of the control) in blood circulation. Wasp venom decreases in the number of RBCs due to its hemolytic activity, and because of this increases the number of hemoglobin level (129.4% of the control) in the plasma of blood.[52] This decreases in the numbers of RBCs in blood circulation causes anemia and circulatory hypoxia.[53] The total hemoglobin content of blood was increased in albino mice treated with *Polistes flavus* venom toxin. This increase may be probably the result of hemoconcentration, which is caused by a massive release of

catecholamines and angiotensin-II.[54,55] Angiotensin-II produces significant decreases in the blood volume and increases in the extravascular fluid.

Wasp venom envenomation increased mean corpuscular hemoglobin (128.9% of the control) in albino mice. This increase in mean corpuscular hemoglobin is due to the hemolysis[56], the number of circulating leucocytes was found to increase (136.3% of the control) after wasp *Polistes flavus* envenomation. Similarly, Yousuf *et al.*, (2003)[57] has reported that honeybee venom toxin injection causes a significant reduction in red blood cells and a frequent elevation total number of leucocytes in mice. The hemoglobin level in blood was significantly increased to maximum level i.e., 101.9%, 103.90%, 107.8% and 122.5% of the control at 2, 4, 6,8, and 10 hour of treatment of 40% of 24-h $LD_{50}$ respectively. The hemoglobin level was increased to maximum 115.6%, 121.5%, and 129.4% of the control at 6, 8, and 10 hour of treatment at 80% of 24-h $LD_{50}$ respectively (Table 2 & 3; Figure 5).

It also caused gradual increase in total number of leucocytes and hemoglobin concentration.[8] It may be caused due to the release of catecholamines by the toxins.[58] However, leucocytosis may be responsible for tissue necrosis, increased secretion of cortisol or both. Increased neutrophil counts indicate systemic inflammatory response related to cytokine release. It may be lead to induction of cytotoxic activity of toxins with hematological and immune alterations.

In experimental mice packed cell volume was increased after envenomation of yellow wasp *Polistes flavus* venom. Wasp venom increases the packed cell volume up to 255% of the control in albino mice (Table 3 & Figure 6). Prominently, melittin causes lyses of erythrocyte cell membrane and also causes reduction in number of red blood cells and elevate the packed cell volume.[4] Melittin is a cationic hemolytic peptide, which bound with negatively charged phospholipids.[59] Besides this, it also makes local conformational changes in the membranes.[40],[60],[42] When the number of red blood cells get decreased due to the effect of wasp venom toxins the adrenergic receptors stimulate the spleen to increase in venous blood flow that leads to increase in blood hemoglobin and packed cell volume.[61]

Yellow wasp *Polistes flavus* venom caused significant ($p<0.05$) reduction in red blood cells and increase in blood hemoglobin, mean corpuscular hemoglobin, packed cell volume, total white blood cells and plasma hemoglobin (Table 2 & 3). Plasma hemoglobin level was increased 111.6% and 115.0% of the control after 10 hours of treatment of 40% and 80% of

24-h $LD_{50}$ respectively (Table 2 & 3; Figure 7). The level of mean corpuscular hemoglobin (MCH) was increased to 123.4% and 128.90% of the control at 8 hours of treatment with 40% and 80% of 24-h $LD_{50}$ of wasp venom. While the level of mean corpuscular hemoglobin decreased to 116.2% and 113.29% of the control after 10 hours of treatment with 40% and 80% of 24-h of $LD_{50}$ of wasp venom toxins (Table 2 & 3; Figure 10). The variation in red blood cells, hemoglobin, MCH, total white blood cells, packed cell volume and plasma hemoglobin was time dependent and dose dependent ($p<0.05$, f-test, student t-test).

In venom administered albino mice rate of hemolysis become very high after 30 minutes of injection. The major reasons are release of extra corpuscular hemoglobin and its direct secretion in urine and displayed as hemoglobinuria. It is a state in which oxygen transport protein hemoglobin exceeds abnormally very high in urine. This condition is also associated with any hemolytic anemia with primarily intravascular hemolysis, in which large number of RBCs is destroyed, thereby releasing free hemoglobin into the plasma. When plasma hemoglobin concentration exceeds the hemoglobin binding capacity of kidney tubular cells get decreased. Therefore, excess free plasma hemoglobin is filtered and excreted in the urine. It results in to acute tubular necrosis and renal failure.[43]

In the present investigation *in vitro* study of RBC hemolysis was also observed at concentration range between 70µg-350µg of *Polistes flavus* venom toxin in albino mice. Hemolysis was time and dose dependent (12.3%-67.7%) (Table 3 & 4, Fig. 11). The number of red blood cells (RBCs) was decreased to 71.0% and 69.8% of the control after 10 hour treatment with 40% and 80% of 24-h $LD_{50}$ of the wasp venom toxins (Table 2 & 3; Figure 8). In vitro effects were established by using five different concentrations of purified yellow wasp *Polistes flavus* venom toxins ranging from 70-350µg were used for hemolytic assays. *In vitro* incubation of RBCs with purified wasp toxin caused dose dependent ($p<0.05$, f-test, student t-test) lysis of red blood cells. The percent hemolysis *in vitro* was found 12.3%, 19.9%, 30.3%, 47.8% and 67.7% at 70µg, 140µg, 210µg, 280µg and 350µg pre-incubation of purified yellow wasp *Polistes flavus* venom respectively (Table 4; Figure 11). The numbers of the total white blood cells (WBCs) was increased to 136.3% and 132.6% of the control after 10 hours of treatment of 40% and 80% of 24-h $LD_{50}$ respectively (Table 2 & 3; Figure 9). The main cause of hemolysis is membrane puncturing and formation of a transient opening by surface bound melittin. However, *in vitro* study it was come out that RBCs were the main target of *Polistes flavus* venom toxins[62] (Table 3). Beside this, massive injection of

wasp venom affects organ system and causes acute renal failure after intra-vascular hemolysis and thrombocytopenia. It also causes an intra-vascular hemolysis, pigment nephropathy and acute renal failure and tubular necrosis.[63],[43] and it also affects cell function and cause necrosis of renal tubular cells.[64] *Polistes flavus* venom envenomation causes malfunctioning of kidney which resulted in elevation in serum creatinine phosphokinase-MM isoenzyme levels suggesting rhabdomylysis.[43]

Reversal of toxic effect was also seen for which antiserum containing polyclonal antibodies was injected in mice that did normalization or improvement of blood parameters. Recovery of physiological and biochemical parameters mainly cells and bio-molecules is a good sign of restoration of physiological conditions.[8] No doubt wasp venom toxins are of very high pharmacological importance.

## CONCLUSION

The results of present study signify yellow wasp venom induced hematological parameters. Venom toxins caused serious dose and time dependent patho-physiological alterations in blood vascular tissues leading to increase in hemolysis, PCV, MCV, MCH, WBCs and hemolglobin level and imposed serious allergic reactions such as inflammation, swelling, nausea in experimental animal. Yellow wasp *Polistes flavus* venom caused significant ($p<0.05$) reduction in red blood cells and increase in blood hemoglobin, mean corpuscular hemoglobin, packed cell volume, total white blood cells and plasma hemoglobin.

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