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In vivo Effects of the Purified Venom of *Polistes flavus* on Blood Bio-Molecules in Albino Mice

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Abstract

In present investigation wasp venom toxins have been isolated from yellow wasp *Polistes flavus* on a gel filtration column-200 B. After administration of sub-lethal dose bio-molecules level such as free amino acids, glucose, pyruvic acids, uric acids and cholesterol were found to be increased but the level of the proteins are decreased in the serum of the albino mice, due to higher oxidation rates that results in a continuous increase in serum free amino acids, glucose, pyruvic acids, uric acids and cholesterol and lowering the protein level. *In vivo* condition, *Polistes flavus* venom toxin caused significant ($p<0.05$) changes in the level of blood bio-molecules such as total protein, free amino acids, glucose, pyruvic acid, uric acid and cholesterol level in the albino mice (*Mus musculus*). The rising level of various bio-molecules in blood serum indicates toxic effects of venom toxins. It happens in response to combat cellular stress and beat toxicity caused by wasp venom toxins.

Keywords: Wasp Venom Toxins; Free Amino Acids; Glucose; Pyruvic Acids; Uric Acids And Cholesterol

Introduction

The hymenopterans are the largest orders among insects, and there are at least 2,00,000 species of bees, wasps and ants. Hymenopterans such as yellow wasps, honeybee, hornets and paper wasps inflict venom or sting for making territorial defense more frequently. Usually, hymenopterans insect toxins generate severe morbidities in organ system. Generally, wasp envenomation occurs after little disturbance in near vicinity of their hive. Wasps inflict venom into the body of enemy by opening their venom apparatus and charge upon heavily for making self-defense. Wasp venom induced toxic effects with multiple organ dysfunction followed by anaphylactic reactions *[1]*. Few people remains non allergic to wasps venoms and show minor local symptoms. But in contrast few person/people show very high allergy to hymenopterans insect venoms and display severe local reactions and systemic symptoms such as anaphylactic reactions. Wasp venom toxins are the immune-system disruptors, neurotoxic, cytotoxic and highly painful with inflammation *[2]*. The important are direct cardio-toxicity, coronary vasospasm, hyperkalemia, pulmonary edema, and hypovolemic shock with internal hemorrhages and intravascular clotting. Anaphylactic effects due to hymenoptera stings are one of the most severe clinical out-comes of IgE-mediated hypersensitivity reactions *[3]* and are related to mild eosinophilia, female sex and concomitant atopic diseases *[4]*. In the severe bite, Na^{+}-K^{+}ATPase pump gets shut off and edema rapidly spreads with extra-vasation of blood, epistaxis and petechie, abdominal pain, paralytic ileus, shock, albuminuria and prolonged clotting time. In multiple stings, the toxin caused severe or even fatal illness.

Arthropod venoms are the rich sources of bioactive compounds which are highly potent chemicals act selectively on multiple molecules targets. These toxins affect the invertebrate's nervous system and until the moment, several insecticidal compounds belonging to the class of peptides or polyamines like compounds have purified and characterized from the venom of arachnids and hymenoptera. Few invertebrate-specific peptides neurotoxins that have been isolated from the venom of spiders, ants, scorpions, wasps etc., shows insecticidal potential against pests and are also invaluable tools in neuro-pharmacology *[5]*. Envenomation by *Nasonia vitripennis* shows developmental arrest, selective apoptosis and alterations in the lipid metabolism in flesh fly hosts *[6]*, while the mastoparan-induced programmed cell death in unicellular alga *Chlamydomonas reinhartii [7]*. The wasp venom is a complex mixture which contains diverse, more or less specific protein components and lethal toxins *[8]*.

Wasp venom toxins are proteinaceous in nature consisting of few peptides, acids, metal bound with protein parts. This toxin possesses a number of charged amino acids that shows multiple biological activities. Other venom components are active amines (serotonin, histamine, tyramine, dopamine, nor-adrenaline and adrenaline), pain producing peptides such as kinins and chemotactic peptides (mastoparans or crabrolin) and proteins including many types of hydrolases (proteases, hyaluronidase, phosphatases, nucleotidases, phospholypases-A) as well as allergens and neurotoxins *[9]*. Venom toxin peptides have 3-6 kDa in size containing between 2,4-disulphide bonds, in a highly stable inhibitors cysteine knot (ICK) motif *[10]*. Major allergens have been identified in

many species of wasps which are of medical importance through a combination of transcriptomic, proteomic, peptidomic, glycomic and venomic approaches *[11]*. Wasp venom contains anti-metagenic substances which induce genotoxic effects *[12]*. Yellow jacket wasp *Polibia poulista* venom peptide (Pp-Hyal) also generates severe allergy in patients *[13]*. It contains hyaluronidase allergen which shows high similarity (97%) with hyaluronidase from *Polistes annularis* venom. Venom proteins contain signature sequences, including reprolysin like dipeptidyl peptidase-4, hyaluronidase, and argine kinase or allergen protein. Allergic reaction to hymenopterans venom occurs in people of any age but it is more common in children and adults and the allergic symptoms occur in 0.3-7.5% of the people *[14-21]*. In present study *Polistes flavus* venom toxins were purified on a gel filtration column and administered in albino mice for visualizing changes in level of certain bio-molecules i.e. total protein, free amino acids, glucose, pyruvic acid, uric acid and cholesterol level in blood.

Material and Methods

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 segments 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 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 venom of yellow wasp *Polistes flavus* was determined.

Determination of the Lethality of Polistes flavus Venom Toxins

The albino mice were injected sub-cutaneously with the purified venom toxins of different serial concentration. The LD_{50} was determined in purified fractions in albino mice 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 Fenney *[22]*. The confidence limits were calculated at 95% probability levels.

Isolation of Blood Serum

Both control and tested albino mice were bled at the same time for obtaining blood serum. Freshly drawn blood was taken directly into a clean glass test tube without adding any coagulants. The blood was allowed to clot in cold. It was centrifuged immediately in a cooling centrifuge at the top speed 15000 rpm for removing any particulate matter from the pellet. lFresh serum was collected and stored at 4°C for experimental purpose. It was used for the analysis of the different biochemical parameters.

Determination of Blood Bio-Molecules

Determination of Serum Total Protein: Estimation of the total protein in the serum of albino mice was carried out by Lowry's method *[23]*. For this, in 0.2 ml of the blood serum 0.3 ml of distilled water was added. In the above mixture added 5.0 ml of freshly prepared alkaline copper solution (Reagent-C/analytical reagent) and allowed the reaction mixture in the room temperature for 15 minutes. After 15 minutes 0.5 ml of (Folin-Ciacalteu) Folin's reagent was added to it. Contents were mixed well and after 15 minutes, a blue color was developed which was measured at 600 nm. The volume of the total protein had been expressed as µg/µl.

Determination of Total Free Amino Acids: Changes in the level of free amino acids in the blood serum of albino mice were determined according to the method of Spies (1957) *[24]*. For this purpose 0.1 ml of blood serum was taken in the glass tube. 0.1 ml of distilled water and 2 ml of the Ninhydrin reagent was added in it and shake well. The mixture was allowed for 15 minutes at boiling water bath. Now cool at room temperature and added 2.0 ml of 50% ethanol. A violet color was developed which was measured at 575 nm. The value of total free amino acid was expressed as µg/µl.

Determination of Serum Glucose: Changes in serum glucose level were measured according to the method of Mendel *et al.*, (1954) *[25]*. For this purpose 0.5 ml of the blood serum was deproteinized by 5%TCA containing 0.1% silver sulphate. The mixture was centrifuged at 10,000 rpm for 10 minutes. In the 0.50 ml of the deproteinized supernatant, 4.5 ml of H_2SO_4 was added and mixed thoroughly. Contents were boiled in water bath for 6 minutes and the mixture was allowed to cool at room temperature. The pink color was developed which was read at the 520 nm. The blank contains only 0.5 ml of 5% TCA containing 0.1% silver sulphate and 4.5 ml of H_2SO_4. The glucose level was expressed as mg/100 ml of blood serum.

Determination of Serum Pyruvic Acid: Changes in the level of pyruvic acid were determined according to the method of Freidman and Haugen (1943) *[26]*. For this purpose blood serum was deproteinized with 5% TCA containing 0.10% silver sulphate and centrifuged at 10,000 rpm for 10 minutes.

Then 1 ml of 2, 4-Di-nitrophenyl hydrazine was added to 0.10 ml deproteinized serum to react at room temperature for 15 minutes. Same procedure was carried out with dilute pyruvic acid standard solution. Now 3 ml of Xylene was added, air was passed and left the mixture for 2 minutes. After setting reaction mixture, the lower layer was discarded by mean of a pipette. Then 6 ml of 10% sodium carbonate was added and mixed again by bubbling the air through the mixture for 2 minutes. After permitting the mixture to settle, 5.0 ml of the aqueous layer was taken in other test tubes and added 5.0 ml of 1.5 N NaOH solutions. It was mixed thoroughly and left for 10 minutes. Absorbance was read at the 520 nm after setting the instrument at zero absorbance with blank containing 5.0 ml of 10% lsodium carbonate and 5.0 ml of the 1.5 N NaOH. The serum pyruvate was measured in terms of mg/100 ml of the blood serum.

Determination of serum uric acids: Changes in serum uric acids level were determined by the Cyanide free method of *Folin [27]*. lIn 1 ml of blood serum added 8 ml of distilled water. After this, 0.5 ml of 0.66N H_2SO_4 added in above mixture. After few minutes 0.5 ml of 10% sodium tungustate solution was added and left stand for 10 minutes to ensure the complete precipitation. Precipitate was filtered and discarded. In 4 ml of filtered solution added 1 ml of 14% (Na_2CO_3) sodium carbonate solution and 1 ml of uric acid reagent and mixture was kept at room temperature for 15 minutes. Absorbance was read at 680 nm setting the instrument to zero density with the solution containing only water and reagent.

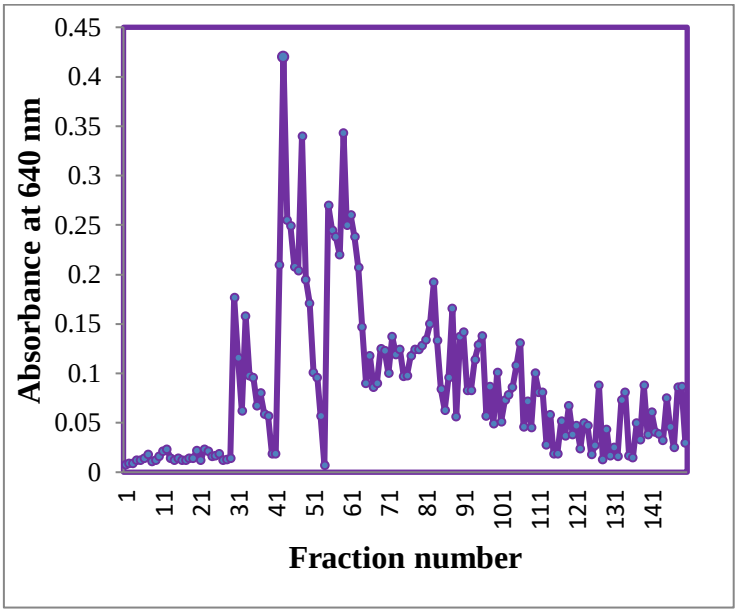
Determination of serum cholesterol: Changes in serum cholesterol level were measured according to the method of *Abell et al. [27]*. In 0.5 ml of serum added 5 ml of alcoholic KOH solution. Contents were shacked well and incubated in a water bath at 37°C for 55 minutes. After 55 minutes allow the mixture to cool at room temperature and added 10 ml of petroleum ether and mixed well. Now added 5 ml of distilled water and shacked vigorously for 1 minute. The contents in mixtures were centrifuged at slow speed (1200 rpm) until two clear layers of petroleum ether and water was obtained. Now 5 ml of aliquot petroleum ether was transferred to a dry test tube and placed in a water bath at 60°C for evaporation of the solvent, a gentle stream of air was blown over the solvent. Then, added 6 ml of Lieberman-Burchard reagents. These test tubes were shacked and returned to water bath. After 30 minutes absorbance were determined at 620 nm.

Standard cholesterol solution was prepared by dissolving 100 mg dry cholesterol in sufficient absolute alcohol to make volume up to 250 ml. This solution contains 0.4 mg cholesterol in 1 ml. A 5.0 ml sample of standard cholesterol solution was mixed with 0.3 ml of 33% KOH solution. The contents were shacked well and incubated in water bath at 37° C for 55 minutes. Contents were cooled at room temperature and added 10 ml of petroleum ether and mixed well. Now added 5 ml of water and shacked vigorously for 1 min. the contents mixtures were centrifuged at slow speed (1200 rpm) until two clear layers of petroleum ether and water was obtained. After centrifugation 1, 2, 3 and 4 ml aliquot of the petroleum ether were taken into four separate test tubes and evaporate to make dry. These standards contained the equivalent 200, 400, 600 and 800 mg of cholesterol/100ml. Now tests tubes were arranged for testing the cholesterol contents in the test tubes and an empty test tubes (for blank) then four tests tubes for standard containing dried sample followed by the unknowns were arranged. Now 5 ml of aliquot petroleum ether was transferred to a dry test tube and placed in a water bath at 60°C. For evaporation of the solvent, a gentle stream of air was blown over the solvent, and then added 6 ml of Lieberman-Burchard reagents. These test tubes were shacked and returned to water bath. After 30 minutes absorbance were determined at 620 nm setting the instrument to read zero density with blank.

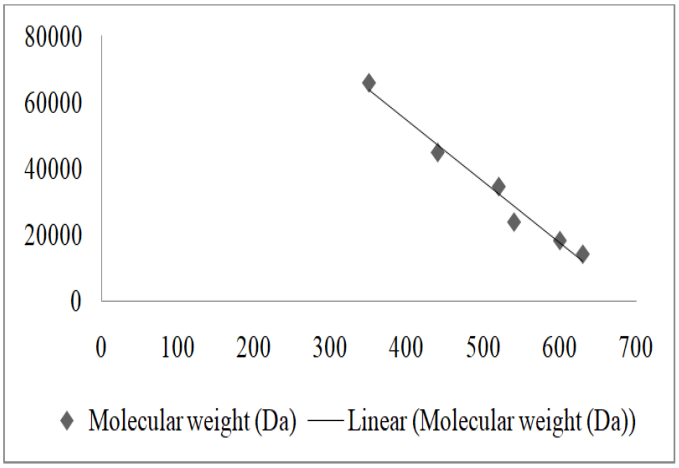
Result

Section 1 l

Sepharose CL-6B 200 Column Chromatography: The elution pattern of purified and homogenized sting glands of yellow wasp exhibited two major peaks at 640 nm, first was major one between the fractions number 46-51 and second a major peak between fractions 51-71. Both peaks were eluted with 0.13M NaCl PBS buffer (pH 6.9) and protein estimation was done for each fraction by Lowry´s method *[23]*. The total yield of protein was 56.23% and specific activity was determined in each fraction (Figure 1).



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. 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 3).

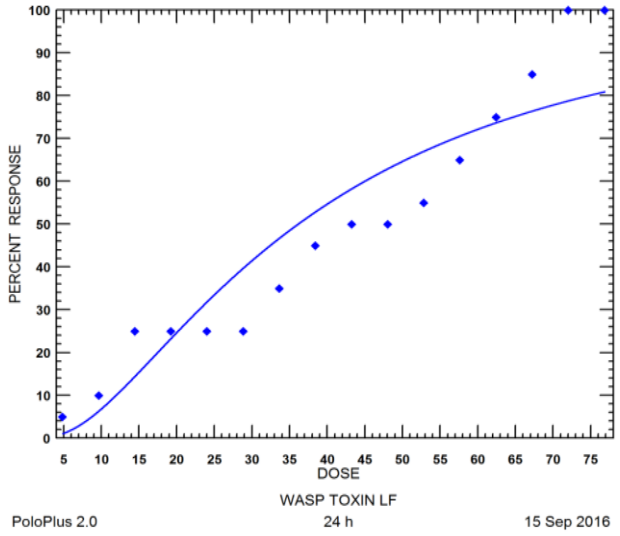


Figure 3: Determination of the LD_{50} of the *Polistes flavus* venom protein in Albino mice by using Mortality was determined by using Probit method's (Fenney, 1971).

Section 2

Polistes flavus venom toxin caused significant ($p<0.05$) changes in the level of blood bio-molecules such as total protein, free amino acids, glucose, pyruvic acid, uric acid and cholesterol level in the albino mice (*Mus musculus*) *in vivo*. The albino mice were treated with the 40% and 80% of 24-h LD_{50} of purified venom toxin of yellow wasp and different parameters were measured after 2, 4, 6, 8 and 10 hours of treatment with respect to control (0-hour). Wasp venom toxin caused significant ($p<0.05$) increased glucose, free amino acids, pyruvic acids, cholesterol and uric acid level in blood serum albino mice, while decreased the level of protein level (Tables 1 & 2).

The serum total protein level was reached at maximum level 132.17% and 140.01% of the control at 40% and 80% of 24-h LD_{50} respectively after 6 hours of treatment. After 10 hours of treatment, the level of protein in serum was decreased to 98.26% and 106.50% of the control at 40% and 80% of 24-h LD_{50} purified venom of *Polistes flavus* (Tables 1 & 2) and (Figure 4) while, the free amino acids level was significantly increased maximum level i.e., 113.60% and 132.50% of the control at 40% and 80% of 24-h LD_{50} after 10 hours of treatment with *Polistes flavus* venom toxin (Tables 1 & 2) and (Figure 5).

Maximum increase in the level of glucose was observed after 10 hours of treatment with purified venom toxins of *Polistes flavus.* After 10 hours of treatment with 40% and 80% of 24-h LD_{50} the level of glucose reached 179.3% and 186.0% in the albino mice in compare to control mice (Tables 1 & 2) and (Figure 6). The pyruvic acid level in blood serum was reached at maximum level 125.0% and 138.33% of the control at 40% and 80% of 24-h LD_{50} after 10 hours of treatment with wasp venom (Tables 1 & 2) and (Figure 7).

The uric acid level in serum was significantly increased at its maximum level i.e., 143.1% and 162.2% of the control at 40% and 80% of 24-h LD_{50} after 8 hours of treatment with *Polistes flavus* venom toxins. The level of uric acid in serum was decreased to 116.6% and 145.5% of the control at 40% and 80% of 24-h LD_{50} after 10 hours of treatment respectively (Tables 1 & 2) and (Figure 8). On the other hand, the level of cholesterol in serum of albino mice was gradually increased and it was reached at maximum level 113.4% and 117.6% of the control at 40% and 80% of 24-h LD_{50} of purified *Polistes flavus* venom toxins after 10 hours of treatment (Tables 1 & 2) and (Figure 9).

The variation in total protein, free amino acid, glucose, pyruvic acid, uric acid and cholesterol level in serum of albino mice after treatment with purified wasp venom toxin was time and dose dependent ($p<0.05$, f-test, student t-test).

Parameters	Time in hours					
	0(Control)	2	4	6	8	10
Protein	2.30±0.081 (100.0)	2.51±0.081 (109.22)	2.72±0.081 (118.2)	3.04±0.081 (132.17)	2.48±0.081 (107.8)	2.25±0.081 (98.26)
Amino acid	1.32±0.081 (100.0)	1.35±0.081 (102.2)	1.38±0.081 (104.5)	1.41±0.081 (106.8)	1.45±0.081 (109.8)	1.50±0.081 (113.6)
Glucose	3.00±0.081 (100.0)	3.20±0.081 (106.6)	3.60±0.081 (120.0)	3.95±0.081 (131.6)	4.75±0.081 (158.3)	5.38±0.081 (179.3)
Pyruvic acid	3.00±0.081 (100.0)	3.10±0.081 (103.3)	3.21±0.081 (107.0)	3.35±0.081 (111.6)	3.54±0.081 (118.0)	3.75±0.081 (125.0)
Uric acid	2.04±0.081 (100.0)	2.22±0.081 (108.8)	2.42±0.081 (118.6)	2.80±0.081 (137.2)	2.92±0.081 (143.1)	2.38±0.081 (116.6)
Cholesterol	2.61±0.081 (100.0)	2.67±0.081 (102.2)	2.75±0.081 (105.3)	2.82±0.081 (108.0)	2.91±0.081 (111.4)	2.96±0.081 (113.4)

Table1: Effects of 40% of 24-h LD_{50} of purified venom toxins of *Polistes flavus* on the bio-molecules such as total protein, free amino acids, glucose, pyruvic acid, uric acid and cholesterol in the albino mice.

Parameters	Time in hours					
	0(Control)	2	4	6	8	10
Protein	2.30±0.081 (100.0)	2.72±0.081 (118.56)	2.96±0.081 (129.10)	3.22±0.081 (140.01)	2.94±0.081 (128.01)	2.45±0.081 (106.5)
Free amino acid	1.32±0.081 (100.0)	1.37±0.081 (103.78)	1.41±0.081 (106.81)	1.49±0.081 (112.80)	1.62±0.081 (122.70)	1.75±0.081 (132.50)
Glucose	3.00±0.081 (100.0)	3.03±0.081 (101.0)	4.02±0.081 (134.0)	4.87±0.081 (162.30)	4.85±0.081 (161.66)	5.58±0.081 (186.00)
Pyruvic acid	3.00±0.081 (100.0)	3.12±0.081 (104.00)	3.40±0.081 (113.33)	3.55±0.081 (118.33)	3.95±0.081 (131.66)	4.15±0.081 (138.33)
Uric acid	2.04±0.081 (100.0)	2.35±0.081 (115.10)	2.45±0.081 (120.00)	3.10±0.081 (151.9)	3.31±0.081 (162.2)	2.97±0.081 (145.50)
Cholesterol	2.61±0.081 (100.0)	2.72±0.081 (104.20)	2.81±0.081 (107.60)	2.95±0.081 (113.00)	3.02±0.081 (115.70)	3.07±0.081 (117.60)

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), *Significant ($p<0.05$, F-test).

Table 2: Effects of 80% of 24-h LD_{50} of purified venom toxins of *Polistes flavus* on the bio-molecules such as total protein, free amino acids, glucose, pyruvic acid, uric acid and cholesterol in the albino mice.

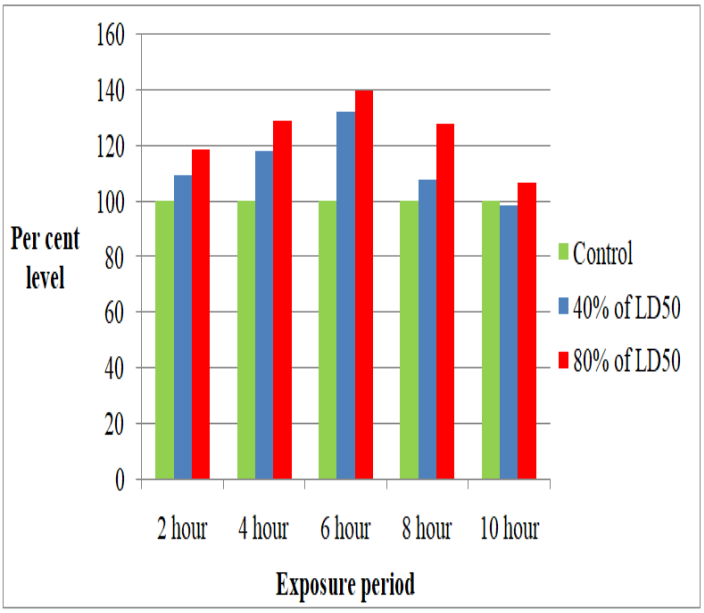


Figure 4: *In vivo* effects of purified venom toxins of yellow wasp *Polistes flavus* on total protein level in serum of the albino mice.

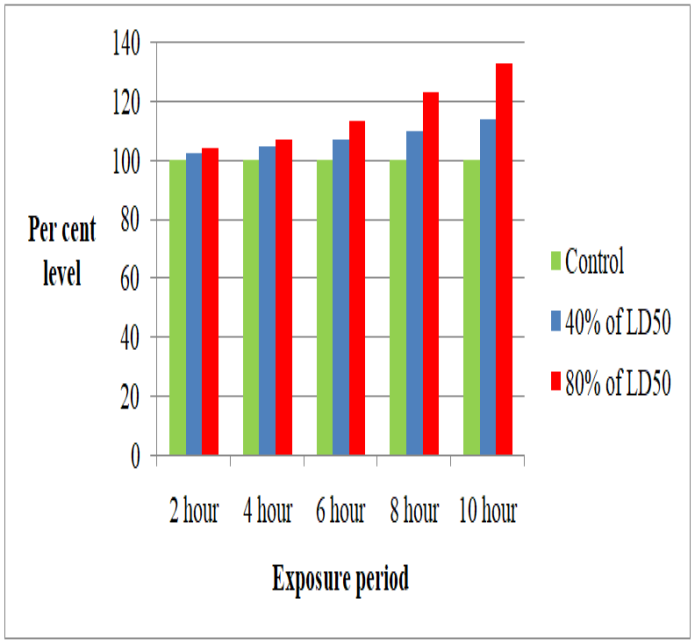


Figure 5: *In vivo* effects of purified venom toxins of yellow wasp *Polistes flavus* on free amino acids level in serum of the albino mice.

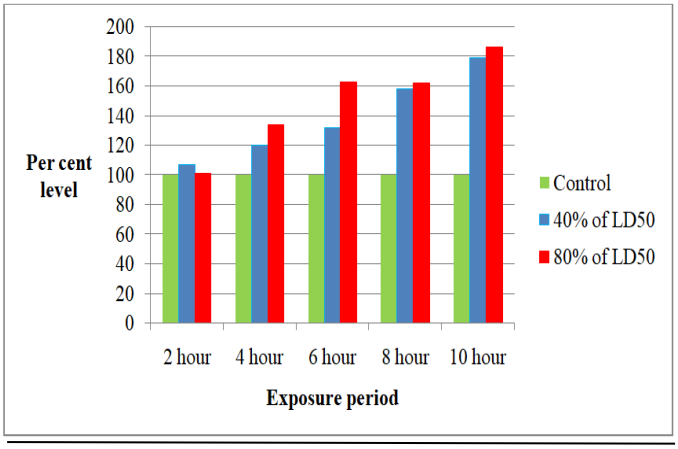


Figure 6: *In vivo* effects of purified venom toxins of yellow wasp *Polistes flavus* on glucose level in serum of the albino mice.

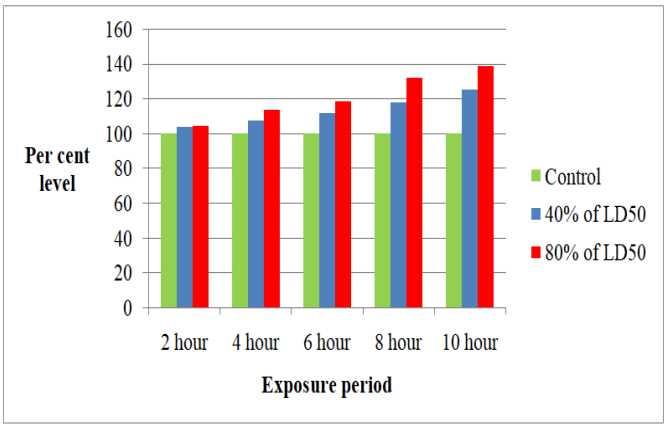


Figure 7: *In vivo* effects of purified venom toxins of yellow wasp *Polistes flavus* on pyruvic acid level in serum of the albino mice.

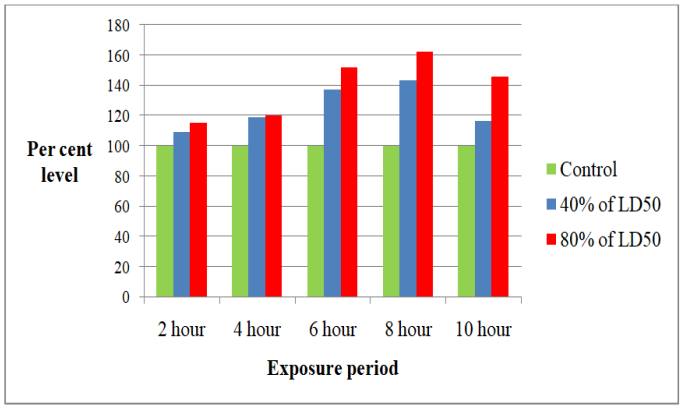


Figure 8: *In vivo* effects of purified venom toxins of yellow wasp *Polistes flavus* on uric acid level in serum of the albino mice.

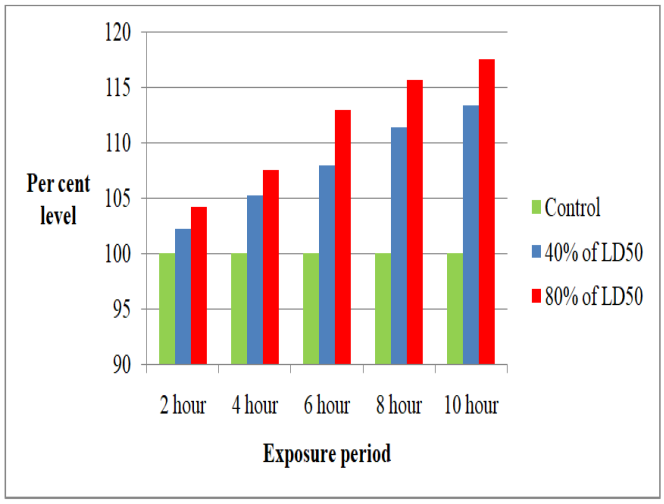


Figure 9: *In vivo* effects of purified venom toxins of yellow wasp *Polistes flavus* on cholesterol level in serum of the albino mice.

Discussion

In each and every year thousands cases of hymenopterans stinging and wasp stinging are reported in the world. It is a major health problem for human beings in rural and urban areas. After feeling a little disturbance in territory or in the close vicinity of hive, wasps reacts and responds very fast to make an attack on predators and mammals. These frequently attack passerby, free dwellers, and children, and inflict venom by the means of stinger *[29,30]*. These inflict venom in to the body of enemy within no time by opening venom apparatus and charge upon heavily for making self-defense.

Yellow wasp *Polistes flavus* venom toxins caused significant ($p<0.05$) increase in lglucose, free amino acids, pyruvic acids, cholesterol and uric acid level in blood serum albino mice. Wasp venom toxins also caused alterations in bio-molecules in albino mice after venom envenomation *[31]*. In the present investigation, level of serum total protein was found to be reduced up to 98.26% at 10^{th} hour in albino mice treated with sub-lethal dose purified *Polistes flavus* venom toxins (Table 1) and (Figure 4). A low total protein level showed a liver disorder or a kidney disorder, or a disorder in which protein is not digested or absorbed properly. Contrary to this, the level of serum free amino acids was found to be elevated 132.50% at 10^{th}hour of the venom injection in comparison to control mice (Table 1) and (Figure 5). This change in *free amino acid level* in blood *serum* of albino mice may be due to spleen deficiency syndrome or due to muscle performance decrements.

Wasp venom toxins also exhibited strong proteolytic activities that cause reduction in serum total protein *[32,33]*. lFurther, it may be due to presence of protease enzymes in wasp venom toxins, which strongly act on protein/peptides and make their conversion into free amino acids and its enhancement *[32]*. Similar proteolytic activity is also seen in social wasp *Polistes infuscatus*, ant *Eciton burchelli [34]* and snake venom toxins *[35,36]* isolated from *Bothrops leucurus* (white tailed jararaca) snake venom. It also showed fibrinolytic activity *[37]*. Besides this, few non-insect poisons also cause significant reduction in the concentration of protein *[38]*. The decline in total protein level with increases in transaminase activity suggests the mobilization of free amino acids during the venom induced stress condition to meet the energy demands *[39]*. Wasp venom toxins also caused a significant ($p<0.05$) increase in serum glucose level i.e., 186.0% at 10^{th} hour (Table 2) and (Figure 6). This is an indication of higher oxidation rate that results in a continuous increase in serum glucose level. Therefore, pyruvic acid level was found to be increased up to 138.3% at 10^{th} hour (Table 2) and (Figure 7). This may be due to massive utilization of glucose for removing the toxic stress. However, hyperglycemia increases the secretion of catecholamines, glucagon, cortisol, thyroid hormones and reduced the less insulin secretion *[40]*. A similar increase in blood sugar was also reported in dogs following envenomation by scorpion venom *Mesobuthus mulusconcanesis [41]*.

Level of blood glucose was found to be elevated after the venom injection in mice *[42]*. Glycogen is the main storage polysaccharides of animal cells and the reason behind this may be more glycogenolysis in stored glycogen in brain, heart, kidney, adipose tissues, and erythrocytes. Another reason might be inhibition of glycogen synthesis due to failure of glucose entrance into the cell through transporters, phosphorylation of glucose to glucose-6-phosphate, isomerization to glucose 1-phosphate, and formation of uridine-5-diphosphate-glucose, which is the direct glucose donor for glycogen synthesis. Glycogen degradation takes place both in the cytoplasm and inside the lysosomes. In the cytosol, glycogen breakdown is accomplished by the coordinated action of two enzymes, glycogen phosphorylase, which releases glucose-1-phosphate by untangling the α1–4 glycosidic linkage and glycogen debranching enzyme that unfastens the branch points releasing free glucose. Similarly, venom fractions from centipede (*Scolopednra moristans*) also causes continuous reduction in glycogen concentrations of intestine; liver, skeletal and cardiac muscles, while level of blood glucose was found to be elevated after the venom injection in mice *[42]*. More specifically, this decrease in stored glycogen level and elevation of blood sugar may be due to toxic stress in test animals. After the entry of toxins in the blood, the animal felt hypoxia and displayed respiratory obstruction. Animal requires more oxygen for catabolism, which can only be compensated by the breakdown of blood glucose with subsequent elevation in pyruvic acid, which convert into acetyl CoA for production of more energy in mitochondria. However, to maintain the blood glucose level, glycogenolysis becomes faster and stored glycogen breaks down *[43]*. Similar, metabolic alteration in human affected with synthetic poison was reported by Yousuf *et al.,* (2003) *[44]*. Wasp venom toxins inhibit the secretion of insulin, a key enzyme in the regulation of carbohydrate metabolism. Infect in animals except brain glucose, at a fixed insulin concentration, promotes its own utilization in a concentration-dependent manner. In turn insulin stimulates glucose oxidation to produce more energy. But low insulin level in blood causes slow glucose oxidation that leads to suppression of lipolysis *[45]*. Therefore, increased level of glucose inhibits the utilization of lipid and increases the level of glucose in blood serum of envenomated mice.

Moreover, serum uric acid level was also found to be increased up to 162.2% at 8^{th} hour of venom injection in comparison to control (Table 2) and (Figure 8). It is evident that uric acid is the end product of purine metabolism, formed from xanthine via a reaction catalyzed by xanthine dehydrogenase. Formation of xanthine dehydrogenase was induced by certain inflammatory cytokines in hypoxia condition *[46]*. In albino mice, serum uric acid are also depends on endogenous synthesis *[47]* and renal excretion *[48]*. These conditions also arises due to dysfunction of mice kidneys, and these don't eliminate uric acid efficiently. There is a possibility that a slow-down in the removal of uric acid is due to effect of toxin component as a diuretic compound. Similarly, in experimental mice treated with sub-lethal dose of the purified *Polistes flavus* venom toxins a marginal elevation i.e. 117.4% in serum cholesterol level was observed at 10^{th} hour (Table 2) and (Figure 9). Wasp venom also caused hyperglycemia in mice leading to elevation in serum glucose and cholesterol level *[49,50]*. The reason behind elevation in the level of cholesterol may *cholesterol in* be elevated sphingomyelin content propose to account for preferential accumulation of the plasma *membrane, or slow rate of cholesterol clearance or desorption* of free cholesterol from *membranes*. It may be due to glycogenolysis and liberation of lipid and cholesterol molecules from membrane disruption. Besides this, intracellular disruption of these molecules may also possible. Another reason of elevation in serum cholesterol may be due to lowering of insulin level *[40]*.

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