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Wasp Venom (*Polistes flavus*) Induced Bio-molecular and Enzymatic Alterations in Albino Mice and Its Reversal After Using Anti-venom

Krishna Kumar Prajapati, Ravi Kant Upadhyay*

Immune Biological Lab, Department of Zoology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, India

Email address:

krishnaprajapati90@hotmail.com (Krishna Kumar Prajapati), rkupadhya@yahoo.com (Ravi Kant Upadhyay)

*Corresponding author

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Abstract: In the present investigation, *in vivo* effects of wasp toxin were evaluated on reversal of metabolic enzymes after providing purified anti-venom antibodies (anti-toxins) at 4 hour of treatment with 40% 24-h LD_{50}. Venom glands of yellow wasp *Polistes flavus* were homogenized and loaded on gel filtration column for purification and isolation of venom toxins/proteins from wasp *Polistes flavus*. These proteins were venom proteins ranging from 14.3-63 kDa. 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. Presence of antibodies in antiserum was tested by using the immune-double diffusion method of Ouchterlony (1962). A precipitin ring was obtained by filling purified antigen and antibody interaction after 24 hrs. Albino mice were treated with 40% of 24-h LD_{50} of purified wasp venom pre-incubated with different doses of purified wasp anti-venom and the neutralizing effects of anti-venom was measured in terms of reversal of metabolic alterations caused by wasp venom, after 4 hours of the treatment. The purified wasp anti-venom significantly ($p<0.05$) reversed the metabolic alterations caused by the wasp venom. The reversal of venom induced metabolic alteration in alkaline phosphatase, acid phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase and acetylcholinesterase activity in the serum of albino mice was dose dependent ($p<0.05$, student t-test). This restoration of enzyme levels in blood serum, liver and gastrocnemius muscles of albino mice also display healing of liver damage, and necrosis in hepatic cells.

Keywords: *Polistes flavus*, Envenomation, Venom Toxins, Metabolic Alterations in Biomolecules, Enzymes, Restoration and Reversal of Toxicity, Immunotherapy

1. Introduction

Wasp stinging is more frequently occurs in rural areas is a common problem and it causes scanty micturation, generalization, swelling and respiratory distress [1]. Wasp venom toxins cause severe inflammation, pain and allergic reaction in farmers, researchers, free dwellers, rural and urban people. In the envenomated patient the sting sites develop, raise red itchy rash and the lesions which are rapidly healed within 3 to 4 days. Physicians, dermatologists, medical and public health entomologists, as well as specific categories of workers should be aware of the risk of exposure to *Sclerodermus* stings [2]. The *Apis indica* venom toxins cause significant serological changes [3], while yellow wasp *Polistes flavus* toxins severely effect blood biochemical parameters and generate toxicity in man [4]. *Nasonia vitripennis* venoms manipulate host immunity and physiology. The venom also leads to changes in behavior in such a way that enhances development of the parasitoid young.

Wasp venom toxins generate multiple organ dysfunction followed by anaphylactic reaction [5]. Some people remains non allergic to wasps venoms and show minor local symptoms. In contrast few persons show very high allergic

reactions to hymenopterans insect venoms and display severe local reactions and systemic symptoms such as anaphylactic reactions. Wasp venom toxins disrupt immune-system, neurotoxic, cytotoxic and highly painful with inflammation [6]. These impose multisystem changes and show wide range biological activities such as intravascular hemolysis, rhabdomylysis, acute renal failure, cardiac, involvement, hepatic dysfunction and occasionally thrombocytopenia and coagulopathy.

Wasp venom toxins impose cardio-toxicity, coronary vasospasm, hyperkalemia, pulmonary edema, and hypovolemic shock with internal hemorrhages and do intravascular clotting. Hymenoptera stings cause anaphylactic effects and induce IgE-mediated hypersensitivity reactions and concomitant atopic diseases [7]. In multiple stings, the venom toxin causes severe or even fatal illness and the Na+-K+ATPase pump gets shut off and edema rapidly spreads with extravasation of blood, epistaxis, abdominal pain, paralytic ileus, shock, albuminuria and prolonged clotting time.

After seeing the causalities caused by wasp stings rural and forest areas there was felt a prompt need of anti-venin to save the life of people. For fast recovery, patients require appropriate treatment, which might be antibody/antisera. Anti-venom is highly demanded and recommended by the clinicians. It can neutralize the effect of venom toxins inside the tissues. No doubt wasp venom toxins are of very high pharmacological importance. Though so many studies have been done on venom of several animals but on the wasp venom toxins, little work has been reported, hence study has been selected for investigation. In the present study wasp toxin have been isolated and purified. Their biological activity has been determined and polyclonal antibodies were raised in albino mice.

2. Material and Method

2.1. Collection of Yellow Wasp and Albino Mice

For isolation of venom toxins, the yellow wasp *Polistes flavus* were collected and 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). The homogenate was centrifuged at 10000 rpm at 4°C for 10 minutes and the supernatant was used as crude venom. For the toxicity testing, six sets of albino mice (*Mus musculus)* were procured from breeding centre; these were of same size, weight and gender. All albino mice were acclimatized in laboratory conditions before setting experiments. Mice were fed on standard pellets, containing all nutritive elements (protein, fats, carbohydrates, vitamins, salts and minerals).

2.2. Preparation of Homogenate

Equal weight of isolated wasp venom glands were homogenized properly in a glass-glass homogenizer in 5 ml of different solubilizing buffers such as Triton X-100, PBS buffer (pH 6.9), 10% TCA, Tris-EDTA and Absolute ethanol separately. Homogenate was centrifuged at 12000 rpm in cold for 30 minutes and supernatant was separated out. Total protein contents were estimated in the different supernatants according to the Lowry's method.

2.3. Purification of Venom Protein of Yellow Wasp

Proteins were eluted on a Sepharose CL-6B-200 a double cavity gel filtration column with sintered disc filtered in the bottom having a height of 1 meter in 25 mm diameter. A 5 ml of venom proteins solubilized in PBS (Phosphate buffer saline) was loaded in the column and the flow rate between 1ml/minute was maintained by a continuous buffer supply in a cold room. Eluted fractions collected at a fixed time interval using test tubes and the values of protein concentration in different eluted fractions were plotted on graph; absorbance in each fraction was determined at 640 nm. Column was tightly held by clips and held erect with stand.

The eluted fractions containing venom protein were pooled and lyophilized to a desired concentration of the venom proteins. The lyophilized venom protein was filled in the dialyzing bag and dialyzed again three changes of phosphate buffer (50mM, pH 6.9) to remove the excess salt from the lyophilized protein venom solution of *Polistes flavus*. Now the pure venom toxins of *Polistes flavus* were used for the experimental work.

2.4. Determination of the Lethality of Polistes flavus Venom Toxins

The albino mice were injected sub-cutaneous 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 noted. 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 [8]. The confidence limits were calculated at 95% probability levels.

2.5. 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 centrifuged at the top speed 15000 rpm for removing any particulate matter from the pellet. Fresh serum was collected and stored at 4°C for experimental purpose. It was used for the analysis of the different biochemical parameters.

2.6. Determination of Biomolecules

2.6.1. Determination of Serum Total Protein

Estimation of the total protein in the serum was carried out

by Lowry's method (1915) [9]. In 0.2 ml of the blood serum added 0.3 ml of distilled water. Add 5.0 ml of freshly prepared alkaline copper solution (Reagent-C/analytical reagent) in it and allowed the reaction mixture in the room temperature for 15 minutes. After 15 minutes 0.5 ml of Folin's reagent (Folin-Ciacalteu reagent) was added in 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.

2.6.2. Determination of Total Free Amino Acid

Changes in the level of free amino acids in the blood serum of albino mice were determined according to the method of Spies [10]. 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 5% ethanol. A violet color was developed which was measured at 575 nm. The value of total free amino acid was expressed as µg/µl.

2.6.3. Determination of Serum Glucose

Changes in serum glucose level were measured according to the method of Mendel *et al.* [11]. For this purpose 0.5 ml of the blood serum was de-protienized by 5% TCA containing 0.1% silver sulphate. The mixture was centrifuged at 10000 rpm for 10 minutes. In this 0.50 ml of the de-protienized 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 room temperature for cooling. 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 Conc. Sulphuric acid (H_2SO_4). The glucose level was expressed as mg/100 ml of blood serum.

2.6.4. Determination of Serum Pyruvic Acid

Changes in the level of pyruvic acid were determined according to the method of Freidman and Haugen [12]. For this purpose blood serum was deproteinized with 5% TCA containing 0.10% silver sulphate and centrifuged at 10000 rpm for 10 minutes. Then 1 ml of 2, 4-Di-nitrophenyl hydrazine was added to 0.10 ml deproteinized serum to react act 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 mixture was left 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 through the mixture for 2 min. 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.

2.6.5. Determination of Serum Uric Acid

Changes in serum uric acid level were determined by the Cyanide free method of Folin [13]. In 1 ml of blood serum added 8 ml of distilled water. 0.5 ml of 0.66N H_2SO_4 added in mixture. After few minutes 0.5 ml of 10% sodium tungustate solution was added and let stand for 10 minutes to ensure complete precipitation. Filtered the precipitation and discarded it. In 4 ml of filtered solution added 1 ml of 14% Na_2CO_3 solution and 1 ml of uric acid reagent and mixture was kept at room temperature for 15 minutes. Read the absorbance at 680 nm setting the instrument to zero density with the solution containing only water and reagent.

2.6.6. Determination of Serum Cholesterol

Changes in serum cholesterol level were measured according to the method of Abell *et al.* [14]. 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 cooling 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. 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 setting the instrument to read zero density with blank.

2.7. Determination of Enzyme Activity

2.7.1. Determination of Alkaline Phosphatase

Changes in alkaline phosphatase level were determined according to the method of Andrech and Szeypiaske (1947) and modified by Bergmeyer [15]. Alkaline phosphate was determined by adding 0.1 ml of enzyme (serum) source to 1 ml of alkaline buffer substrate. The mixture was made up to 100 ml with double distilled water. The incubation mixture was mixed thoroughly and incubated for 30 min at 37°C. After cooling at room temperature added 5 ml of 0.02 N NaOH solutions in incubation mixture. The reaction was stopped by using excess of NaOH. P-nitrophenyl phosphate gave a yellow color with NaOH. Optical density was measured at 420 nm. Standard curve was drawn by using different concentration of p-nitrophenol. Enzyme activity has been expressed as µ moles of p-nitrophenol formed/30 minutes/mg protein.

2.7.2. Determination of Acid Phosphatase

Changes in acid phosphates level were determined according to the method of Andrech and Szeypiaske and modified by Bergmeyer [15]. For estimation 0.2 ml of enzyme source (serum) was taken in a clean test tube and added to it 1.0 ml of acid buffer substance solution was added. The mixture was mixed thoroughly and incubated for 30 minutes at 37°C temperature. After cooling at room temperature added 4.0 ml of 0.1 N NaOH solutions in incubated mixture. A yellow color was developed which was

measured at 420 nm. Standard curves were drawn with P-nitrophenol. Enzyme activity was expressed as µ moles of p-nitrophenol formed/30 minutes/mg protein.

2.7.3. Determination of Serum Glutamate Pyruvate Transaminase (GPT)

Changes in the serum glutamate pyruvate transaminase (GPT) level were measured according to the method of Rietman and Frankel [16]. In this experiment 0.1 ml of blood serum and 0.5 ml of glutamate pyruvate transaminase substrate were added in a clean test tube. The mixture was mixed well and incubated at 37°C for 1 hour. After 1 hour incubation 0.5 ml of 2, 4 di-nitrophenyl hydrazine solutions in incubation mixture was added and incubation mixture was left for 15 minutes at room temperature. After incubation 5 ml of 0.4 N NaOH solution was added in each test tube and contents were allowed for room temperature for 20 minutes. Optical density was measured at 505 nm. Standard curve was prepared by using oxaloacetic acid as working standard. The enzyme activity was measured in unit of glutamate pyruvate transaminase activity/hour/mg protein.

2.7.4. Determination of Serum Glutamate Oxaloacetate Trans-Aminase (GOT)

Changes in the serum glutamate oxaloacetate transaminase (GOT) level were measured according to the method of Rietman and Frankel [16]. In this experiment 0.1 ml of blood serum and 0.5 ml of glutamate oxaloacetate transaminase substrate was added in a clean test tube. The mixture was mixed well and incubated at 37°C for 1 hour. After 1 hour 0.5 ml of 2, 4-di-nitrophenyl hydrazine solution was added and in incubation mixture was left for 15 minutes at room temperature. Then, 5 ml of 0.4 N NaOH solution was added in each test tube and contents were allowed for room temperature for 20 minutes. Optical density was measured at 505 nm. The blank consist of distilled water and 0.4 N NaOH and 2, 4-di-nitrophenyl hydrazine solution and glutamate oxaloacetate transaminase substrate. The enzyme activity was measured in unit of glutamate oxaloacetate transaminase activity/hour/mg protein.

2.7.5. Determination of Serum Lactic Dehydrogenase (LDH)

Changes in the activity of serum lactic dehyrogenase were measured according to the method of Annon [17]. For this purpose, 0.05 ml of blood serum was added to 0.5 ml of pyruvate substrate solution was added to it. The mixture was shake well and incubated at 37°C for 45 minutes. Now 0.5 ml of 0.4 N NaOH solutions were mixed in test tubes and left stand for 30 minutes at room temperature. Optical density was measured at 540 nm and it was converted to lactic dehydrogenase unit by mean of a special prepared standard curve. Enzymatic activity was expressed as µ moles of pyruvate reduced/45 minutes/mg protein.

2.7.6. Determination of Serum Acetylcholinesterase (AChE)

Changes in the activity of the acetylcholinesterase (AChE) were measured according to the method of Ellman [18]. For activity testing 0.05 ml of blood serum and 0.10 ml of acetylcholine thio-iodide solution was mixed in a test tube. To it 0.05 ml of DTNB and 1.45 ml of PBS were added to it and shaked well. Changes in optical density were monitored at 412 nm regularly for 3 minutes at 25°C. Enzyme activity was expressed as µ moles SH hydrolyzed /minute/mg protein.

2.8. Biosynthesis of Polyclonal Antibodies

2.8.1. Choice of Animals

For the biosynthesis of antibodies against *Polistes flavus* venom protein, Albino mice (*Mus musculus*) weighted 65±0.05 gm was used for immunization. These animals were reared in laboratory condition with proper care, feeding and provided treatment humanly for nursing.

2.8.2. Antigen

Purified yellow wasp *Polistes flavus* venom toxins were used as immunogen after mixing with equal amount of Freund's adjuvant.

2.8.3. Dose of Immunization

For primary immunization 50µl (700µg) venom protein and 50 µl of complete Freund's adjuvant were mixed well and injected in the body of albino mice. Immunization was done intra-peritoneal (Figure 1).

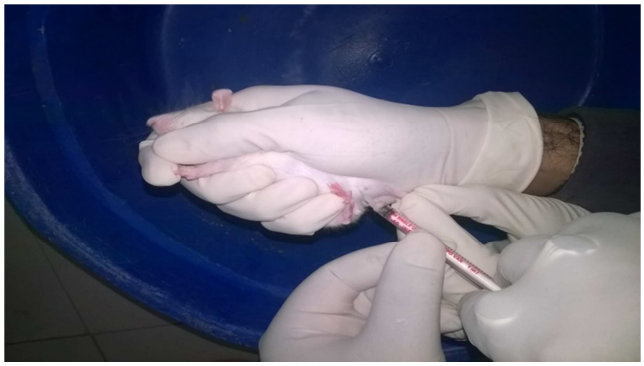

Figure 1. *Immunization of the albino mice (Mus musculus) for raising anti-serum against purified venom of Yellow wasp Polistes flavus. Immunogen was injected subcutaneously.*

2.8.4. Boosting

After 7th days of primary immunization each experimental mice were provided a booster dose (700µg) of venom protein by same route. Similarly a second booster dose was given to the mice after 21th days of primary immunization. After 28th days immunization complete and tested albino mice were ready for antibodies production.

2.8.5. Bleeding and Collection of Blood

After 28th days get the serum from immunization albino mice were bled to obtained serum. For this, freshly drawn blood was collected in the clean glass tube without adding any anti-coagulant and blood was allowed to clot in cold. For obtaining serum, clot was carefully separated by using a clean applicator stick around the inner surface of the tube. It was centrifuged immediately in a cooling centrifuge machine at the 10000 rpm for 10 minutes for removing any particulate

matter and to get clear anti-serum.

2.8.6. Purification of Antibodies and Storage

Anti–serum was partially purified by octanoic acid precipitation and ammonium per sulphate method. For this purpose one volume of anti-serum and two volume of sodium acetate buffer (60 mM) was added at room temperature. To it 1 ml of N-octanoic acid was added drop wise per 10 ml of original anti-serum. Contents were mixed thoroughly for 30 minutes and centrifuged at 1000 x g for 20 minutes and supernatant was taken out. The supernatant was dialyzed against appropriate buffer similar to the dialysis of purified venom.

2.8.7. Detection of the Antibodies in Anti-serum

Presence of antibodies in anti-serum was determined by the method of Ouchterlony [19]. A clean microscopic glass plate was poured with 0.1% agar to make a thin film and allowed to dry. Now the pre-incubated slide was coated with 1% agarose in phosphate buffer-azide solution. One central and four peripheral wells of 3 mm diameter were made on the agar-coated slide. 200µg of anti-serum was added in the central well, while the 200µg of antigen (purified wasp venom) was loaded in peripheral wells. Now this slide was incubated in a humid chamber overnight. After appearance of precipitation band, the glass plate was submerged in 0.15M NaCl for 2 hours to remove non-precipitating protein. The salts were removed from the glass plate by keeping the plate in distilled water. The glass plate was then dried and photographed (Figure 6).

2.8.8. Serotherapy

Efficacy of the anti-venom was tested in albino mice. For this purpose different concentrations (200µg, 400µg and 800µg) of purified polyclonal antibodies were mixed with 40% of 24-h LD_{50}. This mixture was incubated at 37°C for 2 hours and injected in early aged experimental mice having similar body weight 65±0.05 gm each. All the behavioral activities were noted during this period in mice. All important alterations in bio-molecules and enzymes level were determined in the above treated mice after 4 hours on treatment.

3. Result

3.1. Solubilization of Venom Gland of Yellow Wasp

For solubilization of venom toxins/proteins from wasp *Polistes flavus*, different solubilization buffers viz. Triton X-100, Phosphate buffer saline (50 mM, pH 6.9), TCA 10%, Tris-EDTA and absolute alcohol were used. Among which Triton X-100 was proved to be a good solubilizing agent for the *Polistes flavus* venom toxins/proteins. A decreasing order of was obtained in solubilization of venom proteins in different buffers i.e., Triton X-100> Tris-EDTA> TCA 10%> absolute alcohol> PBS (Figure 2).

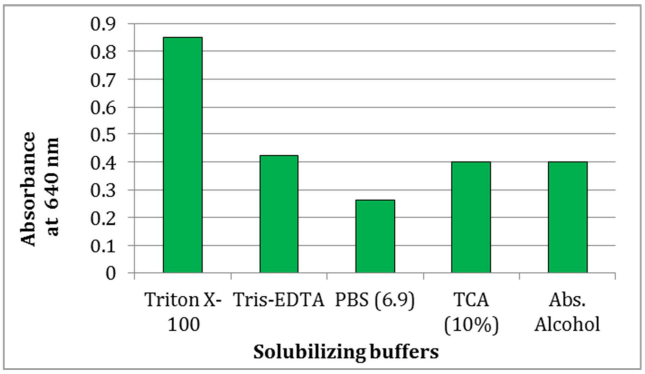

Figure 2. *Solubilization of venom protein of Polistes flavus in different buffers. The absorbance of solubilizing protein was taken at 640 nm. Solubilizing buffers on x- axis are 1. Triton X-100, 2. Tris-EDTA, 3. PBS (6.9), 4. TCA (10%) and 5. Absolute alcohol.*

3.2. Sepharose CL-6B 200 Column Chromatography

The elution pattern of purified and homogenized sting glands of yellow wasp exhibited two major peaks at 280 nm in the fraction no. 41-61 and fraction no. 81-101. These were pooled in separate tubes. Further, protein estimation revealed two protein peaks at 640 nm, first was major one between the fractions no. 46-51 and second a major peak between fractions 60-101. Both peaks were coinciding with first chromatogram obtained at 280 nm. 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. The total yield of protein was 56.23% and specific activity was determined in each fraction (Figure 3).

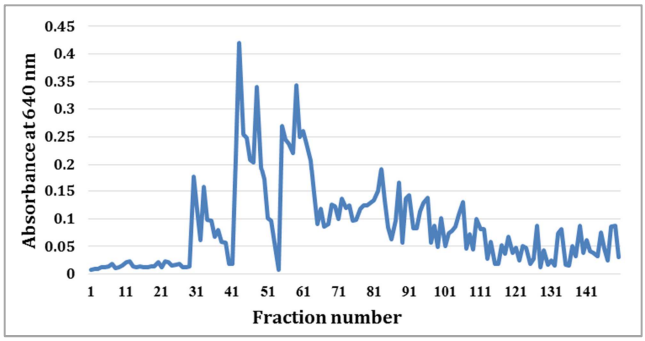

Figure 3. *Elution pattern of phosphate buffer (50 mm, ph 6.9) extractable venom proteins of yellow wasp Polistes flavus chromatographed on Sepharose CL 6B column. Absorbance was taken at 640 nm.*

3.3. 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 4).

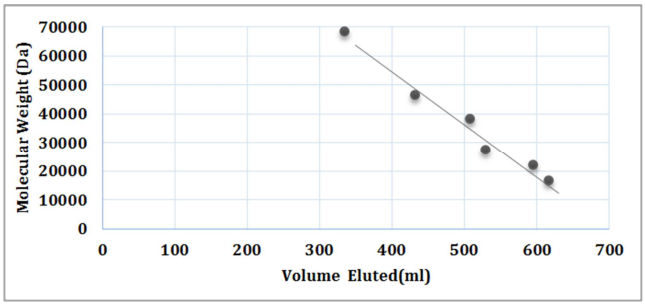

Figure 4. *Standard proteins chromatographed on Sepharose CL-6B 200 gel for determining the molecular weight of venom proteins/peptides isolated from Polistes flavus. Proteins used were bovine albumin mol. wt 66,000, egg albumin mol. wt. 45,000, pepsin mol. wt. 34,700, trypsinogen mol. wt. 24,000, beta lactoglobulin mol. wt. 18,400 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.*

3.4. 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 5).

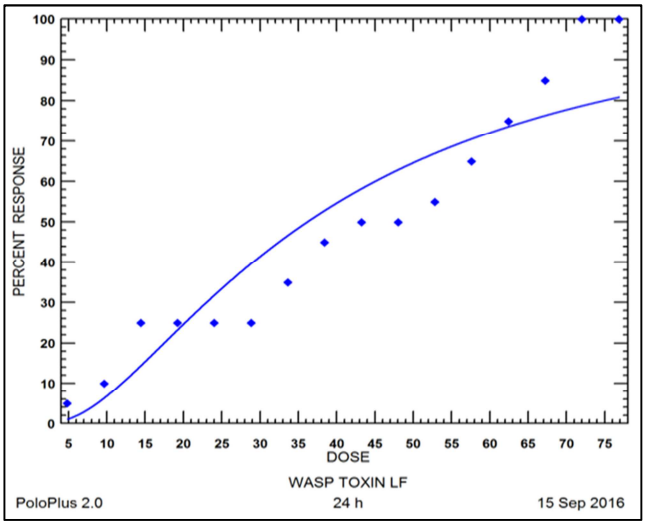

Figure 5. *Determination of the LD_{50} of the Polistes flavus venom protein in albino mice by using mortality was determined by using Probit method's (Fenney, 1971).*

3.5. Detection of Antibodies in Antiserum

Presence of antibodies in antiserum was tested by using the immune-double diffusion method of Ouchterlony (1962). For this purpose both antigen (280 µg) (in the peripheral wells) and antibodies (40 µg) (in the central well) were allowed to interact with each other in humidified gel. By overnight incubation both were diffused radially from their corresponding wells towards each other, thereby establishing concentration gradients. As equivalence zone was reached, a visible bow shaped precipitation band of antigen-antibody complex was formed (Figure 6). In gel when higher concentration of purified antigen (200 µg) and antibody (200 µg) was loaded in central well and peripheral wells and allowed for 24 hours interaction up to a thick precipitin ring was obtained.

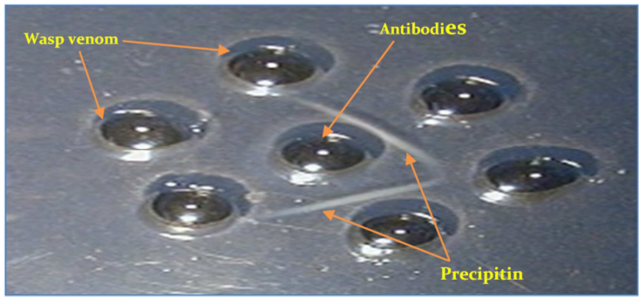

Figure 6. *Immune-double diffusions test for confirmation of antigen-antibody interactions. Central well contains anti-serum and peripheral wells contain antigen. Arrows indicate precipitation bands of antigen-antibody complex.*

3.6. Purification of Antibodies

Anti-venom obtained was treated with Octanoic acid to remove lipoproteins from the anti-serum. Though, this treatment could not concentrate the antibodies but it remained in solution. Further, anti-serum was treated with the ammonium sulphate to precipitate the antibodies and take out them from solution. Thus, ammonium sulphate treatment was used to concentrate the antibodies in the form of pellet. The antibody recovery was 1.01 mg/ml crude anti-serum.

3.7. Reversal of Metabolic Alterations by Purified Wasp Anti-venom

In the study of efficacy of purified wasp anti-venom in the reversal of total protein, free amino acids, glucose, pyruvic acid, uric acid and cholesterol level in the serum of albino mice caused by purified yellow wasp *Polistes flavus* venom toxins. Albino mice were treated with 40% of 24-h LD_{50} of purified wasp venom pre-incubated with different doses of purified wasp anti-venom and the neutralizing effects of anti-venom was measured in terms of reversal of metabolic alterations caused by wasp venom, after 4 hours of the treatment. The purified wasp anti-venom significantly ($p<0.05$) reversed the metabolic alterations caused by the wasp venom.

The experimental mice receiving 40% of 24-h LD_{50} of purified wasp venom pre-incubated with 200µg, 400µg and 800µg of purified wasp anti-venom toxins. They have shown elevation up to 123.33%, 115.00% and 107.50% in total protein level in the serum in comparison to the control mice after 4 hour of treatment, while the mice treated with 40% of 24-h LD_{50} of purified wasp toxin have shown 118.20% of total protein level in the serum in comparison to control mice after 4 hour of treatment (Table 1; Figure 7).

Mice treated with 40% of 24-h LD_{50} of purified wasp venom toxin pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom have shown 103.53%, 101.46% and 98.92% of the free amino acids level in the serum, respectively in comparison to the control mice, while the

mice treated with 40% of 24-h LD_{50} of purified wasp venom showed 104.50% increase in free amino acids level in comparison to control mice after 4 hours (Table 1; Figure 8).

The mice receiving 40% of 24-h LD_{50} of purified wasp venom pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom after 4 hour treatment, have shown 108.57%, 105.71% and 101.42% of the glucose level in comparison to control mice. The glucose level in the mice treated with 40% of 24-h of LD_{50} showed 106.60% of the glucose level in the serum of albino mice (Table 1; Figure 9). Pyruvic acid level in the pre-treated mice with 40% of 24-h LD_{50} of purified wasp venom toxins when pre-incubated with 200µg, 400µg and 800µg anti-venom have shown 104.16%, 102.91% and 101.11% in comparison to control mice after 4 hour of treatment. The pyruvic acid level in the mice treated with 40% of 24-h LD_{50} of purified wasp toxin shown 107.0% in comparison to control mice (Table 1; Figure 10).

The mice treated with 40% of 24-h LD_{50} of purified wasp venom pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom have shown 117.07%, 109.01% and 101.11% of the uric acid level in comparison to control mice after 4 hour treatment, while the mice treated with 40% of 24-h LD_{50} of purified wasp venom shown 118.66% of the control mice after 4 hour of treatment (Table 1; Figure 11).

The mice receiving 40% of 24-h LD_{50} of purified wasp venom toxins pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom had shown 108.21%, 105.11% and 99.52% of the cholesterol level in the serum respectively in comparison to control mice after 4 hour treatment, while the mice treated with 40% of 24-h LD_{50} of purified wasp toxin have shown 109.30% in comparison to control mice after 4 hour (Table 1; Figure 12).

Table 1. *Reversal effect of anti-venom (antibodies) on the level of total protein, free amino acids, glucose, pyruvic acid, uric acid and cholesterol in the serum of albino mice treated with purified wasp Polistes flavus venom toxins.*

Bio-molecules	Doses of anti-venom in µg				
	0µg (control)	4 hour of 40% of LD_{50}	200 µg	400 µg	800 µg
Total protein	1.20±0.012 (100)	1.41±0.012 (118.20)	1.48±0.012 (123.33)	1.38±0.012 (115.00)	1.29±0.012 (107.50)
Amino acid	1.30±0.012 (100)	1.35±0.012 (104.5)	1.34±0.01 (103.53)	1.31±0.01 (101.46)	1.28±0.012 (98.92)
Glucose	3.50±0.014 (100)	3.73±0.014 (106.60)	3.80±0.014 (108.57)	3.70±0.014 (105.71)	3.55±0.014 (101.42)
Pyruvate	2.40±0.08 (100)	2.56±0.08 (107.00)	2.50±0.08 (104.16)	2.47±0.08 (102.91)	2.36±0.08 (101.11)
Uric acid	2.05±0.008 (100)	2.43±0.008 (118.66)	2.40±0.008 (117.07)	2.23±0.008 (109.01)	2.08±0.008 (101.11)
Cholesterol	0.523±0.007 (100)	2.29±0.007 (109.30)	2.27±0.007 (108.21)	2.20±0.007 (105.11)	2.09±0.007 (99.52)

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).

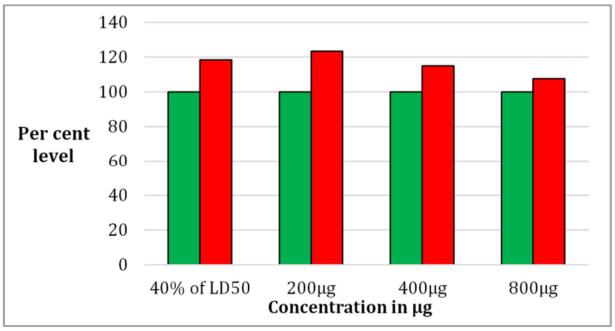

Figure 7. *Reversal of metabolic alterations in the level of total proteins by purified wasp anti-venom after 4 hour of treatment with 40% of 24-h LD_{50}.*

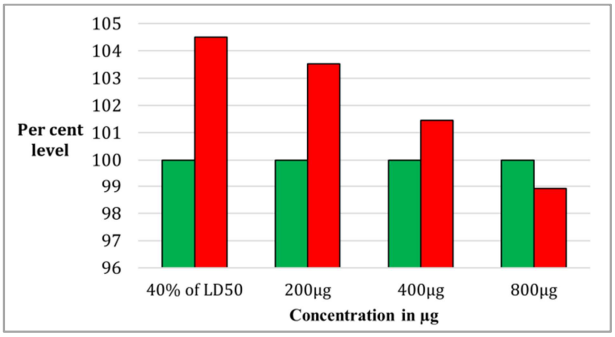

Figure 8. *Reversal of metabolic alterations in the level of free amino acid by purified wasp anti-venom after 4 hour of treatment with 40% of 24-h LD_{50}.*

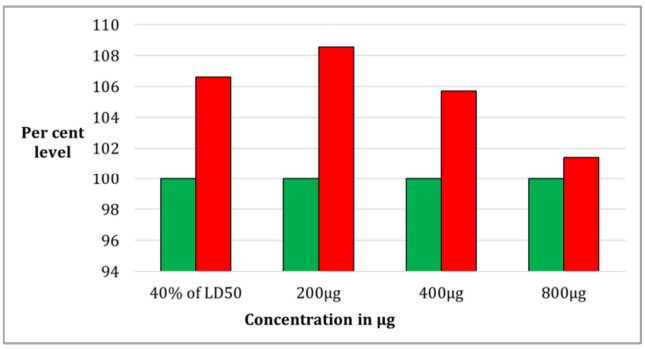

Figure 9. *Reversal of metabolic alterations in the level of glucose by purified wasp anti-venom after 4 hour of treatment with 40% of 24-h LD_{50}.*

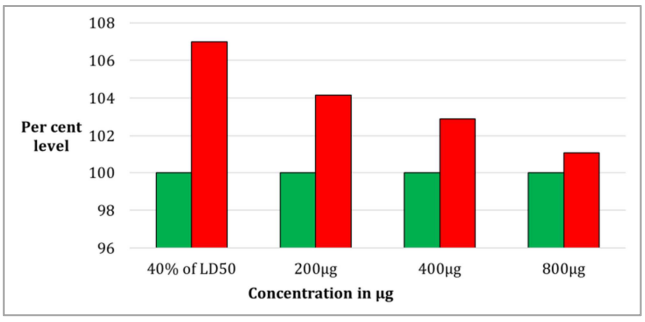

Figure 10. *Reversal of metabolic alterations in the level of pyruvic acid by purified wasp anti-venom after 4 hour of treatment with 40% of 24-h LD_{50}.*

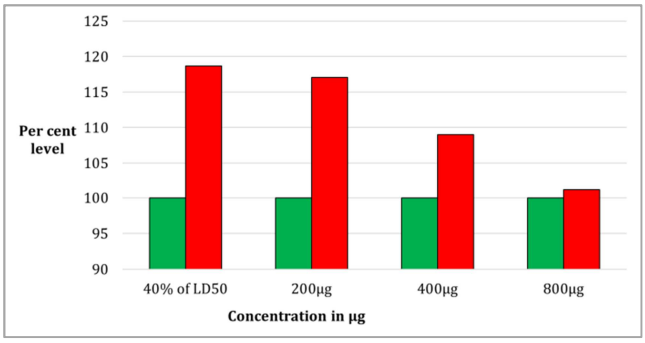

Figure 11. *Reversal of metabolic alterations in the level of uric acid by purified wasp anti-venom after 4 hour of treatment with 40% of 24-h LD_{50}.*

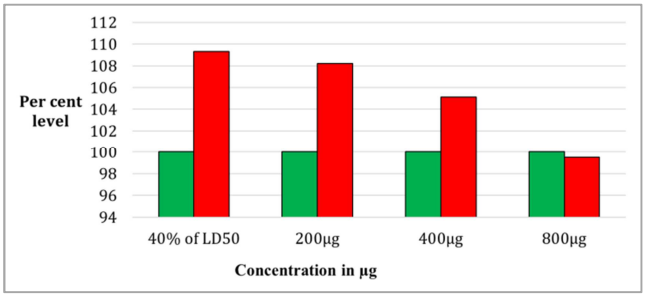

Figure 12. *Reversal of metabolic alterations in the level of cholesterol by purified wasp anti-venom after 4 hour of treatment with 40% of 24-h LD_{50}.*

3.8. Reversal of the Enzymatic Alteration by Purified Wasp Anti-venom

In this section, we study about the efficacy of the purified wasp anti-venom in the reversal of the alteration of the alkaline phosphatase, acid phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase and acetylcholinesterase activity in the serum of albino mice caused by purified venom toxins of the yellow wasp *Polistes flavus*. Albino mice were treated with 40% of 24-h LD_{50} of purified wasp venom toxins pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom. The neutralizing effect of anti-venom was measured in the term of reversal of enzymatic alteration caused by the wasp venom toxins, after 4-hours of the treatment. The purified wasp anti-venom significantly ($p<0.05$) reversed the alterations in the enzymatic activity in the blood serum of mice caused by the wasp venom (Table 2).

The mice treated with 40% of 24-h LD_{50} of purified wasp venom pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom have shown 105.27%, 103.22% and 101.01% of the alkaline phosphatase activity in the serum of mice in comparison to the control, while the mice treated with 40% of 24-h LD_{50} of purified wasp venom have shown 105.15% of alkaline phosphatase activity in comparison to control mice (Table 2; Figure 13).

The mice receiving 40% of 24-h LD_{50} of purified wasp venom pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom have shown 103.51%, 101.12% and 98.56% of the acid phosphatase activity in the serum, respectively in comparison to control mice, while the mice treated with 40% of 24-h LD_{50} of purified wasp venom has shown 103.80% of acid phosphatase activity in comparison to control mice (Table 2; Figure 14).

Treatment with 40% of 24-h LD_{50} of purified wasp venom pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom have shown 135.46%, 120.25% and 105.55% of the glutamate pyruvate transaminase activity in the serum of albino mice in comparison to control, while the albino mice treated with 40% of 24-h LD_{50} after 4 hours of treatment has shown 146.50% in comparison to control mice (Table 2; Figure 15). The mice receiving 40% of 24-h LD_{50} of purified wasp venom toxins pre-incubated incubated with 200µg, 400µg and 800µg purified wasp anti-venom have shown 115.33%, 110.66% and 102.11% of the glutamate oxaloacetate transaminase activity in comparison to control, while the mice treated with 40% of 24-h LD_{50} of purified *Polistes flavus* wasp venom at 4 hours of treatment shown 120.33% of the glutamate oxaloacetate transaminase activity in comparison to control mice (Table 2; Figure 16).

Treatment with 40% of 24-h LD_{50} of purified wasp venom pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom have shown the 103.56%, 101.56% and 99.31% of the lactic dehydrogenase activity in the serum of albino mice respectively in comparison to control mice while the mice treated with 40% of 24-h LD_{50} of purified wasp venom at four shown 104.30% of lactic dehydrogenase activity in comparison to control mice (Table 2; Figure 17). The acetylcholinesterase activity was obtained 97.50%, 98.81% and 99.82% in the mice treated with 40% of 24-h LD_{50} of purified wasp venom pre-incubated with 200µg, 400µg and 800µg purified wasp anti-venom in comparison to control, while the mice treated with 40% of 24-h LD_{50} wasp venom toxin at hour of treatment shown 96.53% of the acetylcholinesterase activity in serum in comparison to control mice (Table 2; Figure 18).

Table 2. *Reversal effect of anti-venom antibodies (anti-toxins) on alkaline phosphatase, acid phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase and acetylcholinesterase activity in the serum of albino mice treated with purified wasp Polistes flavus venom toxins.*

Enzymes	Doses of anti-venom in µg				
	0 µg (control)	4-hour of 40% of LD_{50}	200 µg	400 µg	800 µg
ALP	251.05±0.8 (100)	263.97±0.8 (105.15)	264.28±0.8 (105.27)	259.13±0.8 (103.22)	253.85±0.8 (101.01)
ACP	281.85±0.8 (100)	292.56±0.8 (103.80)	291.71±0.8 (103.51)	285.00±0.8 (101.12)	277.79±0.8 (98.56)
GPT	1.975±0.014 (100)	2.895±0.014 (146.50)	2.675±0.014 (135.46)	2.374±0.014 (120.25)	2.084±0.014 (105.55)
GOT	1.283±0.08 (100)	1.543±0.08 (120.33)	1.479±0.08 (115.33)	1.419±0.08 (110.66)	1.310±0.08 (102.11)
LDH	2.62±0.008 (100)	2.732±0.008 (104.3)	2.713±0.008 (103.56)	2.66±0.008 (101.56)	2.60±0.008 (99.31)
AChE	1.252±0.007 (100)	1.208±0.007 (96.53)	1.220±0.007 (97.50)	1.237±0.007 (98.81)	1.249±0.007 (99.82)

Blood serum was the enzyme source; Values are mean ± SE of three replicates; Values in parentheses indicates percentage activity with respect to control taken as 100%; *Significant ($p<0.05$, Student t-test); *Significant ($p<0.05$, F-test); Alkaline phosphatase (ALP): µ moles of p-nitrophenol formed/30 minutes/mg protein; Acid phosphatase (ALP): µ moles of p-nitrophenol formed/30 minutes/mg protein; Glutamate pyruvate transaminase (GPT): Units of Glutamate pyruvate transaminase activity/hour/mg protein; Glutamate oxaloacetate transaminase (GOT): Units of Glutamate oxaloacetate transaminase activity/hour/mg protein; Lactic dehydrogenase (LDH): µ moles of pyruvate reduced/45 minutes/mg protein; Acetylcholinesterase (AChE): µ moles 'SH' hydrolyzed/minute/mg protein.

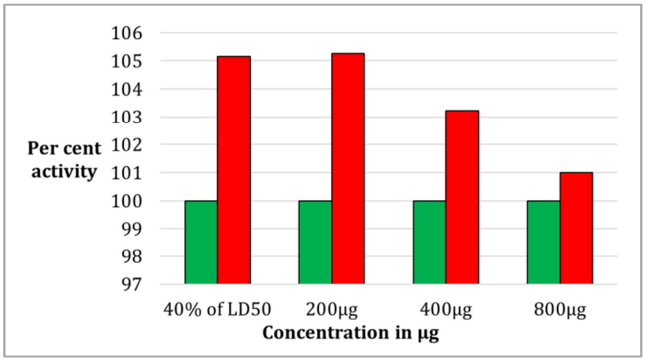

Figure 13. *Reversal of metabolic alterations in the alkaline phosphatase activity by purified anti-venom antibodies (anti-toxins) at 4 hour of treatment with 40% 24-h LD_{50}.*

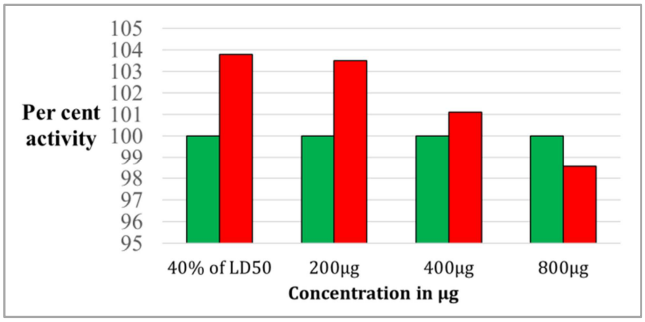

Figure 14. *Reversal of metabolic alterations in acid phosphatase activity by purified anti-venom antibodies (anti-toxins) at 4 hour of treatment with 40% 24-h LD_{50}.*

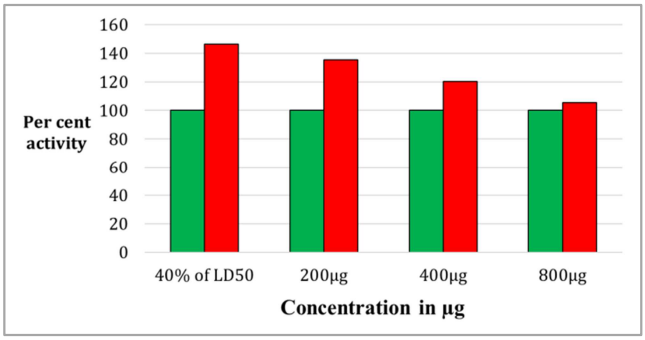

Figure 15. *Reversal of metabolic alterations in glutamate pyruvate transaminase activity by purified anti-venom antibodies (anti-toxins) at 4 hour of treatment with 24-h LD_{50}.*

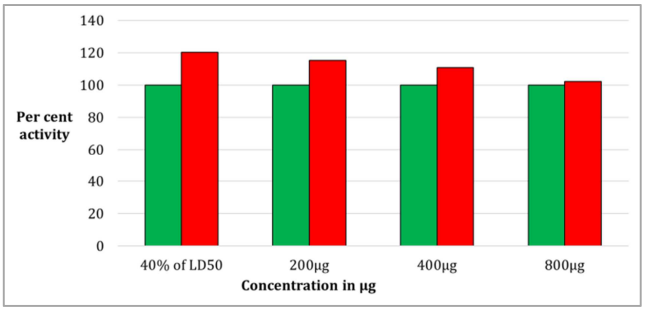

Figure 16. *Reversal of metabolic alterations in glutamate oxaloacetate transaminase activity by purified anti-venom antibodies (anti-toxins) at 4 hour of treatment with 24-h LD_{50}.*

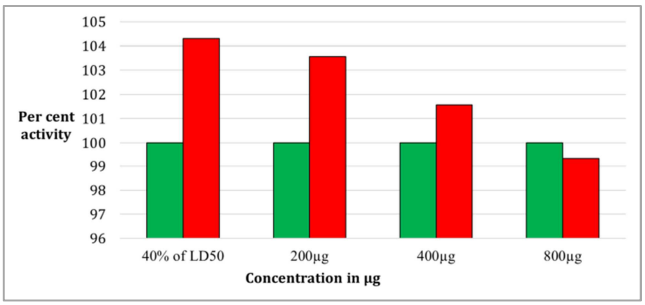

Figure 17. *Reversal of metabolic alterations in lactic dehydrogenase activity by purified anti-venom antibodies (anti-toxins) at 4 hour of treatment with 40% 24-h LD_{50}.*

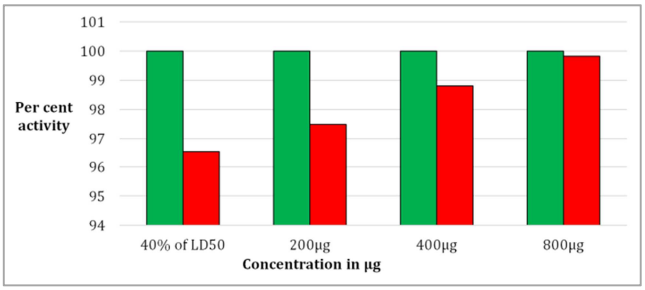

Figure 18. *Reversal of metabolic alterations in acetylcholinesterase activity by purified anti-venom antibodies (anti-toxins) at 4 hour of treatment with 40% of 24-h LD_{50}.*

4. Discussion

In present study, *Polistes flavus* venom toxins were isolated and purified for production of polyclonal antibodies in mice model for establishing toxicity neutralization and reversal effects. Due to lack of information on the anti-serum therapy the present investigation was designed to investigate the various pharmacological effects due to wasp venom in albino mice. More emphasis was given on evaluation of the potential effects of the wasp venom on blood biochemistry of the albino mice to observe the pharmacological effects. The aim of the present study was to determine the possible alterations in biochemical parameters in large and small animals with FMD and under the effect of wasp venom. Hence, from the various bioassays it was confirmed that *Polistes flavus* venom is highly toxic to mammals. It significantly altered the level of bio-molecules, metabolic

enzymes activities.

Venom protein of *Polistes flavus* was tested for its solubility in different solvents and maximum solubility was observed in Triton X-100 (Figure 2). Wasp sting apparatus with venom glands were isolated and homogenized in PBS (pH 6.9) and purified on Sepharose CL-6B 200 gel filtration column chromatography. Chromatograms resolved in two major peaks at 280 nm in the fraction no. 41-61 and fraction no. 81-101. These were polled in separate tubes (Figure 3). Further concentration and fractionation of venom proteins again revealed two peaks at 640 nm, a minor one between the fraction no. 46-51 and a major peak between fractions 60-101. 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. The total yield of protein was 56.23% and specific activity was determined in each fraction. Molecular weight of the purified *Polistes flavus* venom toxins were rinsed from 14.3-63 kDa (Figure 4). The LD_{50} of the wasp venom protein was found 36.11 mg/kilogram body weight i.e., 0.03611 mg/gram body weight of albino mice (Figure 5).

Reversal effect of anti-venom antibodies and restoration of physiological parameters and animal behavior for nullifying the lethal effect of wasp venom polyclonal antibodies were generated in albino mice. For this purpose purified (*Polistes flavus*) venom toxins were mixed with Freund's adjuvant and injected in to the experimental mice intra-peritoneal. After seven days booster dose was provided. Similarly a 2^{nd} booster was also given to the mice after 21 days (Figure 1). Mice were bled for collection of blood after four weak. Clot was broken to get the antiserum. The presence of polyclonal antibodies in the antiserum was detected by method of Ouchterlony (1962). Due to antigen and antibody interaction an equivalence zone was formed, which is appeared in the form of a visible band after precipitation. This precipitation band represents the formation of antigen-antibody complex (Figure 6).

In the present investigation neutralization of wasp venom toxins was also observed. For this purpose different concentrations (200µg, 400µg, 800µg) of polyclonal antibody were mixed and incubate with 40% of 24-h LD_{50}. This pre-incubated mixture was injected in experimental mice. It was found that all physiological effects were normalized and anti-venom reversed the oxidative stress and other adverse effects such as muscular paralysis, hypotension and allergic responses in experimental mice. Further, metabolic and enzyme alterations in the blood serum of the mice were also found reversed after 4 hr antibody treatment. Similarly efficacy of honeybee anti-venom has also been tested by Jones *et al.* [20]. However, the efficacy of anti-scorpion venom serum in patients stung by scorpion *Buthusta mulus* by exploring the data of reversed metabolic reactions [21].

4.1. Effects on Bio-molecules

Yellow wasp *Polistes flavus* venom toxins caused significant ($p<0.05$) increase in glucose, free amino acids, pyruvic acids, cholesterol and uric acid level in blood serum albino mice. Wasp venom toxin also causes alterations in bio-molecules in albino mice after a massive envenomation [22]. 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. Changes in serum free amino acid level in patients with spleen deficiency syndrome or due to muscle performance decrements. It suggests that wasp venom toxins also exhibit strong proteolytic activities that make reduction in serum total protein [23, 24]. Paper wasp *Polistes flavus* and other insects such as social wasp *Polistes infuscatus*, ant *Eciton burchelli* [25], it may be due to presence of protease enzymes in wasp venom, which strongly act on protein and peptides and make their conversion into free amino acids [23]. Besides this, few non insect poisons also cause significant reduction in the concentration of protein [26]. The decline in total protein level with increase in transaminase activity suggests the mobilization of free amino acids during the venom induced stress condition to meet the energy demands [27].

Moreover, 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 [28]. Albino mice serum uric acid is also depends on endogenous synthesis [29] and renal excretion [30]. These condition also arise 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. Wasp venom also caused hyperglycemia in mice leading to elevation in serum glucose and cholesterol level [31, 32]. The reason behind elevation in the level of cholesterol may be elevated sphingomyelin content propose to account for preferential accumulation of cholesterol in the plasma membrane, or slow the rate of cholesterol clearance or destoration of free cholesterol from the membrane. 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 [33].

Wasp venom toxins also caused a significant ($p<0.05$) increase in serum glucose level and 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. Contrary to this, level of glycogen was found to be decreases in liver, gastrocnemius and cardiac muscles. 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 [33]. A similar increase in blood sugar was also reported in dogs following envenomation by scorpion venom *Mesobuthusta mulusconcanesis* [34].

Most of the physiological and biochemical alterations were found to restore after 8 hours of venom injection. This recovery may be due to the facts that subcutaneous

administration of the venom requires at least 8 hours for the complete absorption from the injected site. This recovery indicates that the alterations caused by the venom toxins at low doses are not permanent but are temporary. However, at higher doses of the wasp venom toxins all the above changes will reach beyond the limit in tolerance of the envenomated mice, which cannot be recovered easily and leading to the death of experimental mice.

4.2. Effect on the Activity of Certain Metabolic Enzymes

In the present investigation activity of certain metabolic enzymes were also found to be altered after injection of sub lethal dose of purified *Polistes flavus* venom toxins to the albino mice. A significant elevation was observed in serum acid phosphatase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and lactic dehydrogenase activity, while a significant ($p<0.05$) reduction was observed in acetylcholinesterase activity. However, it is well known that liver synthesize metabolic enzymes and stored them for catabolic activity. However, wasp venom toxins disintegrate liver cells and cause liver intoxication. Due to disintegration, most of the enzyme leaks out from liver and muscle cells into the circulation [35]. Besides this, both acid phosphatase and alkaline phosphatase enzymes are also considered as detoxifying enzymes and their level increased in human poisoning [36]. These enzymes are mainly found in blood plasma, liver and intestine of human beings [37, 38].

Acid phosphatase is the lysosomal enzyme that plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis [39]. *Polistes flavus* venom toxins also cause liver ischemia and hypoxia, which resulted in increase in level of serum acid phosphatase [40]. On the other hand, alkaline phosphatase is an important membrane bound enzymes found in all body tissues. It mediates the transport of metabolites across the membrane. It also plays an important role in protein synthesis [41]. increase in activity of alkaline phosphatase may retard the protein synthesis in tissues and release excess free amino acids in to the circulation, thereby, increasing amino acid level in the serum. Venom toxins isolated from *Gymnapistes marmoratus* (Soldier fish) have displayed higher level of serum acid phosphates, alkaline phosphatase and phosphodiesterase in human victims [42]. This elevation in ACP might be due to increase in lysosomal disintegration and ALP is due to hemolytic activity of the venom.

Glutamate pyruvate transaminase (GPT) plays a key function in carbohydrate metabolism. It makes a way for the delivery of skeletal muscle alanine to the liver. In skeletal muscle pyruvate is transaminated to alanine and transported to the liver. Inside liver glutamate pyruvate transaminase transfers the ammonia to the Alpha-ketoglutarate and regenerates pyruvate. This pyruvate is then utilized in the process of gluconeogenesis for glucose production [43]. Therefore, it functions as a link between carbohydrate and protein metabolism by catalyzing the conversion of alanine to pyruvate. Glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and lactic dehydrogenase are cellular metabolic enzymes with no evident function in vertebrate plasma. These enzymes also occur in small concentrations in plasma, which may be delivered from the regular physiological shedding of cells [44]. Therefore, any detectable increase in their activity in plasma can be used as a reliable indicator of changes in metabolic functions and structural damage in tissues [45]. *Polistes flavus* venom caused massive cellular toxicity in liver cells and caused significant alterations in cell permeability of myocardial, liver and smooth muscle cells, which may facilitate the release of certain metabolic enzymes out of the cells in to the circulation. The elevation in GPT activity may be due to stress that was created after *Polistes flavus* venom injection. Therefore, venom induced stress may be the causative factor for the elevation in GPT concentration [35].

It is evident that during stress condition the energy requirement becomes high, which results in very high utilization of glucose and massive breakdown of stored glycogen that leads to decrease in glycogen level [43]. Similarly, Egyptian scorpion venom after administration of 100-400 µg/kg dose of lyophilized venom caused a significant increase in serum glucose, creatinine, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and lactic dehydrogenase activity in mice [46]. Besides LDH, AST or ALT levels are a valuable aid primarily in the diagnosis of liver disease. Although not specific for liver disease, it can be used in combination with other enzymes to monitor the course of various liver disorders.

Therefore, elevation in LDH level increases the glucose catabolism for energy production especially in anaerobic condition. Besides this, increased level of LDH in muscle and liver cells shows insufficient oxygen supply. It is well known that pyruvic acid is the main end product of glycolysis in those tissues, which are supplied oxygen in abundance, but in those tissues where oxygen supply is insufficient or in anaerobic state. Example skeletal muscles lactic acid form the usual end product of glycolysis [34]. In such cases pyruvic acid is reduced to lactic acid under the influence of lactic dehydrogenase.

It clearly indicates binding of certain venom components with this enzyme. This inhibition of acetylcholinesterase activity causes accumulation of acetylcholine molecules at the synaptic junctions. This accumulation of acetylcholine may lead to prolonged activation of acetylcholine receptors and a permanent stimulation of nerves and muscle cells resulting in muscular paralysis and finally death of animal. (This inhibition of acetylcholinesterase activity causes accumulation of acetylcholine molecules at the synaptic junctions. This accumulation of acetylcholine may lead to prolonged activation of acetylcholine receptors and a permanent stimulation of nerves and muscle cells resulting in muscular paralysis and finally death of animal [47].

Finally, polyclonal antibodies were generated against purified venom toxins of yellow wasp *Polistes flavus* in albino mice. Anti-venom was purified by octanoic acid and ammonium sulphate treatment and the presence of antibody

in antiserum was detected by immuno-double diffusion method given by Ouchterlony. The venom toxin and antibody interaction gave a cris-centric appearance at the junction of antigen and antibody interaction. Observing all of the physiological alterations after the anti-venom therapy tested the efficacy of purified anti-venom. For this purpose, 40% and 80% of 24-h of LD_{50} of wasp venom was pre-incubated with different concentrations (200µg, 400µg and 800µg) of purified anti-venom and this pre-incubated venom was injected intraperitoneally to the mice. Therefore, the parameters under observation were measured at 4th hour of the treatment. In the experiments it was found that all abnormalities regarding bio-molecules and enzymes in the blood serum of mice were found to be completely reversed after the anti-venom treatment. Hence, it can be concluded that polyclonal antibodies produced against wasp (*Polistes flavus*) are clinically more effective and may be more usable for wasp stung patients.

5. Conclusion

From the above studies it is clear that wasp venom toxins put adverse effect on body metabolism and alter biochemical functions of important bio-molecules such as proteins, amino acids, glucose, pyruvic acid, uric acid and cholesterol. Wasp venom also caused hyperglycemia in mice leading to elevation in serum glucose and cholesterol level. 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. Wasp venom toxins also caused a significant ($p<0.05$) increase in serum glucose level and this is an indication of higher oxidation rate that results in a continuous increase in serum glucose level.

Wasp venom toxins have been significantly altered the level of important metabolic enzymes i.e. alkaline phosphates, acid phosphates, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase and achetylcholinesterase. A significant elevation was observed in serum acid phosphatase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and lactic dehydrogenase activity, while a significant ($p<0.05$) reduction was observed in acetylcholinesterase activity. Molecular weight of the purified *Polistes flavus* venom toxins were rinsed from 14.3-63 kDa. The LD50 of the wasp venom protein was found 36.11 mg/kilogram body weight i.e., 0.03611 mg/gram body weight of albino mice. Purified *Polistes flavus* venom toxins were used for production of polyclonal antibodies in mice model. The presence of polyclonal antibodies in the antiserum was detected by method of Ouchterlony (1962). Due to antigen and antibody interaction equivalence zone was formed, which is appeared in the form of a visible crecentric precipitin band due to antigen and antibody interaction and formation of its complex. These were injected pretreatment and post treatment for establishing toxicity neutralization and reversal effects. Presence of antibodies in antiserum provided positive effects as the toxins were successfully neutralized by anti-serum, and all pharmacological effects evoked due to wasp venom in albino mice were reversed and restored after 8 hrs of administration of antiserum in experimental mice. This neutralization of toxins effects by polyclonal antibodies has great clinical significance for stung patients.

Conflicts of Interest

The authors declare that they have no competing interests.

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