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Effects of Purified Paper (Yellow) Wasp (*Polistes flavus*) Venom Toxins on Phosphatase Enzyme Activity in Blood Serum, Liver and Gastrocnemius Muscle Tissue of Albino Mice

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Abstract: In this study biological effects of purified wasp venom toxins were evaluated on phosphatase enzyme activity in blood serum, liver and gastrocnemius muscle tissue of albino mice. A significant elevation was observed in serum acid phosphatase, alkaline phosphatase in serum, liver and gastrocnemius muscles in albino mice after injection of sub-lethal dose of purified *Polistes flavus* venom toxins.

Polistes flavus venom toxins also cause liver ischemia and hypoxia, which resulted in increase in level of serum acid phosphatase and alkaline phosphatase, may retard the protein synthesis in tissues and release excess free amino acids into the circulation, thereby, increasing amino acid level in the serum. Both conditions clearly indicate toxic effects of venom toxins on membrane and muscle cell functions.

Keywords: Intoxication, liver ischemia and hypoxia, detoxifying enzymes, ALP, ACP

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Introduction

There are two fundamental conditions for the life of organisms. First, the animal must be able to self-replicate and second it must be able to catalyze the chemical reactions efficiently and selectively. Wasp venom toxins generate multiple organ dysfunction followed by anaphylactic reaction (Xie *et al.*, 2013). These impose multisystem changes and show

wide range of biological activities such as intravascular hemolysis, rhabdomyolysis, acute renal failure, cardiac involvement, hepatic dysfunction and occasionally thrombocytopenia and coagulopathy. The sting sites developed red itchy rash and the lesions on the human beings rapidly healed within 3-4 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 (Papini *et al.*, 2014). Envenomation in-group are highly fatal to humans as it causes severe inflammation, swelling, rhabdomyolysis, renal-insufficiency and severe pain. After few seconds of envenomation, toxins cause heavy RBCs hemolysis and damage nerve cells and inhibit biochemical functions of enzymes and proteins. It also causes allergic reactions by immune stimulation of the body (Cummins *et al.*, 2006). In general stinging hymenopterans are active participants in social defense that have greatly influenced the relationship. Predators have been a strong component of the selection pressure in the evolution of painful and toxic bee, wasp, and ant stings and these insects, in turn, have influenced hunting behavior and learning in at least higher primates (Schmidt *et al.*, 2014). Hymenopterans venom had evolved and through different mechanisms manipulates host immunity and physiology. The venom of wasp also leads to change in behavior in such a way that enhances development of the parasitoid young. The venom from the ectoparasitoid *Nasonia vitripennis* inhibits the immune system in its host organism in order to protect their offspring from elimination (Danneels *et al.*, 2014).

The venom toxin of *Polistes flavus* contains some important enzymes i.e., phospholipase-A, hyaluronidase, acid phosphatase and D-glycosidase which are highly antigenic in nature. The hyaluronidase acts as a spreading factor that allows the toxic substances to infiltrate the tissues and rupture the blood cells. Phospholipase-A shows no general hypersensitivity and toxicity to the tissues but

indirectly inhibits action of thrombokinase, dehydrogenase and transaminase and also inhibits oxidative phosphorylation (Kettner *et al.*, 2001). The phospholipids from the *Polistes flavus* venom usually do not contain carbohydrates and have highly homologous region of active sites. The phospholipids of the wasp venom digest the cell wall components of di-acylphospholipids such as phosphotidylcholine, phosphotidylserine, phosphotidyl ethanolamine to fatty acids and lysophospholipids with PLAs. The phospholipase-B is more universally digestive enzymes than PLA1 and PLA2 and this is present in the venom of paper wasp *Polistes flavus*. Toxins are special biological substances which are produced and inflicted by organism to make self-defense. The defensive symbiont *Hamiltonella defensa* which protects aphids against attacks by parasitoid wasps is one of these conditional mutualists (Olivier *et al.*, 2014).

The principal wasp venom enzymes are phospholipase-A2, acid phosphatase and mono-esterase. These enzymes indirectly inhibit activity of certain other enzymes i.e., thrombokinase, dehydrogenase, transaminase (Betten *et al.*, 2006). The phospholipases show haemolytic activities and cardio-toxicity in experimental animals (Abe *et al.*, 2000). Few wasp venom toxins are enzymes phospholipases-A₁, hyaluronidases and acid phosphatases which show vasoactive and thrombotic activity (Piek *et al.*, 1982). Several other peptides have been identified with antibacterial properties (Saidenberg *et al.*, 2010). Heavy envenomation induces wasp venom allergy (WVA), and severe life-threatening cardiopulmonary collapse with breathing difficulties, bronchospasm, hypotension and arrhythmia (Piek *et al.*,

1986). IgE-level increase after the sting causes symptomatic sensitization (Sturm *et al.*, 2013). Mast cells (MC) are effectors cells during severe systemic reaction (SR) to hymenoptera stings. Tryptase and Prostaglandin D₂ metabolites (PGDs) are the markers of MC activation (Dias *et al.*, 2015).

Acid phosphatase is a major allergen in paper wasp venom and its availability as recombinant protein may facilitate the development of improved diagnostic tests and immuno-therapies for the envenomated patients. Venom toxins generate strong T-cell responses in hypersensitive patients and interact with IgE-antibody molecules. APImb can be used in diagnosis and therapy of bee venom toxicity (Quistad *et al.*, 1994). APImb also signifies production of specific IgE antibodies after envenomation. The venom of social wasp evolved to be used as defensive tools to protect the colonies against attacks of predators. Wasp venom comprises altogether up to 70% of the weight of freeze dried venoms (Daury *et al.*, 1997). The wasp venom is a complex mixture that contains diverse, more or less specific protein components (Burke *et al.*, 2014). Wasp venom consists of high molecular weight enzyme molecules ranging from 15.0-50.0 kDa. In present investigation effects of purified wasp venom toxins were evaluated on phosphatase enzyme activity in blood serum, liver and gastrocnemius muscle tissue of albino mice.

Materials and Methods

Isolation of venom protein from paper wasp: The living paper wasp *Polistes flavus* were collected from different region of Gorakhpur, India. They were immobilized by quick freezing at -20 C. The venom reservoir i.e., venom glands were taken out by last segment of abdomen region of wasp and homogenized

in phosphate buffer saline (PBS) (50mM, pH 6.9) with the help of power homogenizer. The homogenate was centrifuged at 3000 g at 4 C for 5 minutes and the supernatant was used as crude venom.

Preparation of gel filtration column:

Gel filtration column of double cavity with sintered disc in the bottom having a height of 1 meter and 25 mm in diameter was used for separation and isolation of *Polistes flavus* venom toxins. The dead space inside the elution front was kept to minimum. The loading front was kept closed with a rubber cork. All the accessories required for gel filtration column were assembled according to Speir (1982).

Fraction collection:

Eluted fractions of paper wasp *Polistes flavus* venom proteins were collected manually at a fixed time interval. Elution patterns of the venom proteins through gel filtration column were done at the flow rate of 5 ml/minutes.

Spectrophotometric observation and protein estimation of the eluted fraction:

The eluted fractions were observed for the detection of presence of venom protein at a wavelength of 280 nm. A graph was plotted between absorption at 280 nm and fraction numbers to show the elution pattern of paper wasp *Polistes flavus* venom protein. This process was repeated for confirmation of the detection of presence of venom protein at a wavelength of 640 nm. The protein content eluted in each fraction was determined by using the method of Lowry *et al.* (1951).

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 $V_e/V_o \log M$ and with the help of calibration curve range of molecular weight of different protein in the purified paper wasp *Polistes flavus* venom was determined.

Isolation of blood serum, liver and gastrocnemius muscles from albino mice:

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 15000 rpm for removing any particulate matter from the pellet. Fresh serum was collected and stored at 4 °C for experimental purpose. After collecting the blood serum, liver and gastrocnemius muscles were dissected out from albino mice and were used for the analysis of the alkaline and acid phosphatase enzyme activity.

Determination of total protein in serum, liver and gastrocnemius muscles:

Estimation of the total protein in the serum was carried out by Lowry's method (1915). 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-Ciocalteu) was added in it. Contents were mixed well and after 15 minutes a blue color was developed which was measured at 600 nm. Estimation of the total protein in the liver, gastrocnemius and

heart muscles were carried out by Lowry's method (1915). For this purpose 100 ml of tissues were homogenized in 10% TCA and centrifuged it 10000 rpm for 10 minutes. The supernatant was used as protein source. The volume of the total protein had been expressed as $\mu\text{g}/\mu\text{l}$.

Determination of alkaline phosphatase in serum:

Changes in alkaline phosphatase level were determined according to the method of Andrech and Szeypiaske (1947) and modified by Bergmeyer (1967). Alkaline phosphatase 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 the incubated mixtures at room temperature, added 5 ml of 0.02 N NaOH in incubation mixture. The reaction was stopped due to excess of NaOH. P-nitrophenyl phosphate gave a paper 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.

Determination of alkaline phosphatase in liver and gastrocnemius muscles:

Changes in alkaline phosphatase (ALP) level were determined according to the method of Andrech and Szeypiaske (1947) and modified by Bergmeyer (1967). For this purpose 100 mg of tissue was homogenized in 1.0 ml of 0.9% NaCl solution and centrifuged at 5000 g for 15 minutes in a cooling centrifuge. The supernatant was used as enzyme source and further processed similar to method described

earlier. 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 the mixture at room temperature then added 5 ml of 0.02 N NaOH in incubation mixture. The reaction was stopped due to excess of NaOH. P-nitrophenyl phosphate gave a paper 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.

Determination of acid phosphatase in serum: Changes in acid phosphatase level were determined according to the method of Andrech and Szeypiaske (1947) and modified by Bergmeyer (1967). In this method, 0.2 ml of enzyme source (serum) was taken in a clean test tube and added 1.0 ml of acid buffer substance solution. The mixture was mixed thoroughly and incubated for 30 minutes at 37 C. After cooling at room temperature added 4.0 ml of 0.1 N NaOH solutions in incubated mixture. A paper color was developed which was measured at 420 nm. Standard curves were drawn with P-nitrophenol. Enzymes activity was expressed as μ moles of p-nitrophenol formed/30 minutes/mg protein.

Determination of acid phosphatase in liver and gastrocnemius muscles:

Changes in acid phosphatase level were determined according to the method of Andrech and Szeypiaske (1947) and modified by Bergmeyer (1967). For this purpose 100 mg of tissue was homogenized in 1.0 ml of 0.9% NaCl solution and centrifuged at 5000 x

g for 15 minutes in a cooling centrifuge machine. The supernatant was used as enzyme source and in 0.2 ml of enzyme source (serum) was taken in a clean test tube and added 1.0 ml of acid buffer substance solution. The mixture was mixed thoroughly and incubated for 30 minutes at 37 C. After cooling at room temperature added 4.0 ml of 0.1 N NaOH solutions in incubated mixture. A paper color was developed which was measured at 420 nm. Standard curves were drawn with p-nitrophenol. Enzymes activity was expressed as μ moles of p-nitrophenol formed/30 minutes/mg protein.

Results

Purification of venom protein:

The venom glands of the paper wasp *Polistes flavus* were homogenized in PBS buffer and centrifuged at 10,000 rpm in cooling centrifuge machine and the supernatant was used as crude venom and lyophilized at desired concentration. The elution pattern of purified and homogenized sting glands exhibited two major peaks at 280 nm in fraction no. 41 - 71 and 81 - 101 (Fig. 1). Further at the 640 nm the elution pattern of purified venom protein exhibited two major peaks between 41-51 and 51-71 fractions numbers and both peaks were eluted with 0.13 M NaCl PBS buffer (pH 6.9) and protein estimation was done for each fraction by using Lowry's method (Fig. 2). The total yield of protein was 69.21% and specific activity was determined in each fraction (Fig. 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

Fig. 1: Elution patterns of phosphate buffer (50 mM, pH 6.9) extractable venom proteins of paper wasp *Polistes flavus* chromatographed on Sepharose CL-6B 200 column. Absorbance was taken at 280 nm.

Fig. 2: Elution pattern of phosphate buffer (50 mM, pH 6.9) extractable venom proteins of paper wasp *Polistes flavus* chromatographed on Sepharose CL-6B 200 column. Absorbance was taken at 640 nm.

Fig. 3: Both peaks were eluted with 0.13M NaCl PBS buffer and protein estimation was done for each fraction by using Lowry method.

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

standard marker proteins of known molecular weight. Each elution fraction having 5 ml content and the major peaks were obtained in the 41-71 fraction numbers. So, the calibration curve indicates that the molecular weight of purified venom proteins ranging from 14.3 to 63 kDa (Fig. 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 paper wasp venom proteins obtained from the lyophilization of the two peaks caused toxicity in the albino mice. The LD₅₀ of the paper wasp *Polistes flavus* venom protein was found 36.11 mg/kg body weight i.e., 0.03611 mg/g body weight of albino mice (Fig. 5).

The toxic effect of the purified venom toxins of *Polistes flavus* were observed in albino mice on serum, liver and gastrocnemius muscles enzyme activity of alkaline phosphatase and acid phosphatase. The albino mice were treated with 40% and 80% of 24 h LD₅₀ of purified wasp toxins and alterations in enzyme activity were measured after 2, 4, 6, 8 and 10 h of treatment. Wasp venom caused significant increase in the activity of alkaline phosphatase and acid phosphatase activity in serum, liver and gastrocnemius muscles in treated albino mice in comparison to control mice (Tables 1, 2).

Fig. 5: Determination of the LD₅₀ of the *Polistes flavus* venom protein in Albino mice by using Probit method (Fenney, 1971).

The alkaline phosphatase activity was significantly increased in the serum of albino mice and reached to 112.72% and 138.19% of the control mice at 6 h of treatment of 40% and 80% of 24 h LD₅₀ of paper wasp venom toxins. While the ALP activity was gradually decreased to 105.50% and 113.02% of the control mice at 10 h treatment with 40% and 80% of 24 h LD₅₀ of paper wasp venom toxins (Tables 1, 2; Figs. 6, 7). The activity of alkaline phosphatase (ALP) was significantly increased in liver tissues and reached to 141.38% and

144.18% of the control mice at 10 h after treatment with 40% and 80% of 24 h LD₅₀ of paper wasp venom toxin and in gastrocnemius muscles the ALP activity was increased to 119.15% and 127.51% of the control mice at 10 h after treatment with 40% and 80% of 24 h LD₅₀ of paper wasp venom toxin (Tables 1, 2; Figs. 6, 7).

The acid phosphatase (ACP) activity was significantly altered in the blood serum, liver and gastrocnemius muscles in albino mice treated with 40% and 80% of 24 h LD₅₀ of paper wasp venom toxin. The acid phosphatase activity in serum was significantly increased to 106.0% and 107.34% at 6 h treatment and after 10 h treatment the ACP activity was gradually decreased to 95.25% and 99.92% of 40% and 80% after treatment with 24 h LD₅₀ of paper wasp venom as compared to control mice (Tables 3, 4; Figs. 8, 9). In the liver the ACP activity was significantly increased to 111.29% and 113.28% of the control mice at 10 h after treatment with 40% and 80% of 24 h LD₅₀ of *Polistes flavus* venom protein (Tables 3, 4; Figs. 8, 9). In this experiment when albino mice were treated with venom toxin of paper wasp *Polistes flavus*, the variation in the alkaline phosphatase and acid phosphatase activity in the serum, liver and gastrocnemius muscles of albino mice after treatment with purified venom of *Polistes flavus* showed time- and dose-dependent response ($p < 0.05$, Student's t-test).

Table 1: *In vivo* effects of 40% of 24 h LD₅₀ of purified venom toxins of *Polistes flavus* on the activity of alkaline phosphatase in serum, liver and gastrocnemius muscles

Tissues	Time (h)					
	0 (Control)	2	4	6	8	10
Serum	133.16±0.08 (100.0)	136.5±0.08* (102.50)	140.03±0.08 (105.15)	150.01±0.08* (112.72)	145.10±0.08* (108.96)	140.5±0.08* (105.50)
Liver	131.91±0.8 (100.0)	133.63±0.8 (101.30)	136.90±0.8* (103.78)	147.40±0.8* (111.74)	162.40±0.8* (123.11)	186.50±0.8* (141.38)
Gastrocnemius Muscles	61.03±0.08 (100)	62.09±0.08 (101.73)	64.00±0.08* (104.86)	67.87±0.08* (111.20)	70.21±0.08* (115.04)	72.72±0.08* (119.15)

Values are mean ± SE of three replicates

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

*Significant (p<0.05, Student's t-test)

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

Blood, liver and G. muscles the enzyme source.

Alkaline phosphatase (ALP): μ moles of p-nitrophenol formed/30 min/mg protein.

Fig. 6: Alkaline phosphatase activity in serum, liver and gastrocnemius muscles in albino mice treated with 40% of 24 h LD₅₀.

Fig. 7: Alkaline phosphatase activity in serum, liver and Gastrocnemius muscles in albino mice treated with 80% of 24 h LD₅₀.

Table 2: *In vivo* effects of 80% of 24 h LD₅₀ of purified venom toxins of *Polistes flavus* on the activity of alkaline phosphatase in serum, liver and gastrocnemius muscles

Tissues	Time (h)					
	0 (Control)	2	4	6	8	10
Serum	133.16±0.08 (100.0)	161.5±0.08 (121.28)	181.03±0.08* (135.94)	184.02±0.08* (138.19)	167.10±0.08* (125.48)	150.5±0.08* (113.02)
Liver	131.91±0.8 (100.0)	138.40±0.8* (104.90)	141.70±0.8* (107.42)	152.60±0.8* (115.68)	176.90±0.8* (134.10)	190.20±0.8* (144.18)
Gastrocnemius Muscles	61.03±0.08 (100)	63.81±0.08 (104.55)	65.82±0.08* (106.10)	69.52±0.08* (113.91)	72.76±0.08* (119.22)	77.86±0.08* (127.51)

Values are mean ± SE of three replicates

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

*Significant (p<0.05, Student's t-test)

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

Blood, liver and G. muscles the enzyme source.

Alkaline phosphatase (ALP): μ moles of p-nitrophenol formed/30 min/mg protein.

Fig. 8: Acid phosphatase activity in serum, liver and Gastrocnemius muscles in albino mice treated with 40% of 24 h LD₅₀.

Table 3: *In vivo* effects of 40% of 24 h LD₅₀ of purified venom toxins of *Polistes flavus* on the activity of acid phosphatase in serum, liver and gastrocnemius muscles

Tissues	Time (h)					
	0 (Control)	2	4	6	8	10
Serum	135.37±0.08 (100.0)	137.12±0.08 (101.30)	140.51±0.08* (103.80)	143.49±0.08* (106.0)	138.23±0.08* (102.11)	128.95±0.08* (95.25)
Liver	182.13±0.08 (100.0)	184.43±0.08 (101.26)	186.83±0.08* (102.58)	189.90±0.08* (104.26)	195.22±0.08* (107.18)	202.71±0.08* (111.29)
Gastrocnemius Muscles	187.92±0.08 (100)	199.00±0.08* (105.89)	211.82±0.08* (112.34)	216.88±0.08* (115.41)	222.76±0.08* (118.53)	227.77±0.08* (121.20)

Values are mean ± SE of three replicates

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

*Significant (p<0.05, Student's t-test)

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

Blood, liver and G. muscles the enzyme source.

Acid phosphatase (ACP): μ moles of p-nitrophenol formed/30 min/mg protein.

Fig. 9: Acid phosphatase activity in serum, liver and Gastrocnemius muscles in albino mice treated with 80% of 24 h LD₅₀.

Table 4: *In vivo* effects of 80% of 24 h LD₅₀ of purified venom toxins of *Polistes flavus* on the activity of acid phosphatase in serum, liver and gastrocnemius muscles

Tissues	Time (h)					
	0(Control)	2	4	6	8	10
Serum	135.37±0.8 (100.0)	133.60±0.8 (98.69)	134.23±0.8 (99.15)	145.35±0.8* (107.34)	133.91±0.8 (98.92)	135.27±0.8 (99.92)
Liver	182.13±0.08 (100.0)	186.71±0.08 (102.51)	188.43±0.08* (103.45)	192.47±0.08* (105.67)	199.23±0.08* (109.38)	206.33±0.08* (113.28)
Gastrocnemius Muscles	187.92±0.08 (100)	201.42±0.08* (107.18)	213.43±0.08* (113.57)	219.28±0.08* (116.68)	225.73±0.08* (120.12)	236.05±0.08* (126.23)

Values are mean ± SE of three replicates

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

*Significant (p<0.05, Student's t-test)

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

Blood, liver and G. muscles the enzyme source.

Acid phosphatase (ACP): μ moles of p-nitrophenol formed/30 min/mg protein.

Discussion

In the present investigation *Polistes flavus* venom proteins or toxins were isolated and purified by gel filtration column chromatography by using Sepharose CL-6B

200 as gel matrix. Ahmad *et al.* (2011) have also solubilized the venom proteins isolated from venom glands of the Indian honey bees *Apis indica* in phosphate buffer and Jones *et al.* (1999) have also solubilized the venom

proteins isolated from venom glands of the African honey bees *Apis mellifera* in phosphate buffer.

The elution patterns of purified and homogenized sting glands of paper wasps exhibited two major peaks at 280 nm in the fraction no. 41-71 and fraction no. 81-101. These were pooled in separate tubes. Further concentration and fractionation of venom proteins again revealed two peaks at 640 nm, major one between the fraction no. 41-51 and second 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. The total yield of protein was 56.23% and specific activity was determined in each fraction. Moreover, molecular weight of wasp *Polistes flavus* venom was also determined on gel filtration chromatography. Venom proteins showed molecular weights ranging from 14.3-63 kDa between the 41-71 fractions and many peaks were observed in chromatograms shows presence of many peptides. Haim *et al.* (1999) characterized venom peptides from *Vespa orientalis*. The median lethal dose (LD₅₀) of the yellow wasp *Polistes flavus* venom protein was found 36.11 mg/kg body weight i.e., 0.03611 mg/g body weight of albino mice.

In the present investigation activity of ALP and ACP enzymes was 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 the activity of acid phosphatase and alkaline phosphatase. 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 (Bouck *et al.*, 1966). Both the acid and alkaline phosphatase enzymes are also considered as detoxifying enzymes and their level increased in human poisoning (Srinivas *et al.*, 2003). These enzymes are mainly found in blood, liver, plasma and intestine of human beings (Arkhypova *et al.*, 2001; Luskova *et al.*, 2002).

In the albino mice, activity of alkaline phosphatase was found to be increased up to 112.72% and 138.19% at 6 h in comparison to control mice and the activity of serum ALP was decreased to 105.50% and 113.02% at 10 hafter treatment with 40% and 80% of 24 h LD₅₀. However, the ALP activity in liver and gastrocnemius muscles was increased to 144.18% and 127.51% at 10 h. This elevation may be due to cytolysis. Contrary to this the activity of alkaline phosphatase was found to be increased up to 144.18% and 127.51% in liver and gastrocnemius muscle at 10 h, respectively in comparison to control. This increase in activity of alkaline phosphatase may retard the protein synthesis in tissues and release excess free amino acids into 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 (Hopkins *et al.*, 1998). Similarly, frequent elevation was noted in alkaline and acid phosphatase in turkey hens after a dose of 1,2,4-triazole derivative (3-(2-pyridil)-4phenyl-1, 2,4-triazole-5-carboxylic acid) (Krauze *et al.*, 2007).

On the other hand, the activity of serum acid phosphatase was increased up to 107.34% at 6 h in comparison to control mice. The acid phosphatase is the lysosomal enzyme

that plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis (Abou-Donia *et al.*, 1978). *Polistes flavus* venom toxins also cause liver ischemia and hypoxia, which resulted in increase in level of serum acid phosphatase (Abraham *et al.*, 1967). Similarly, in liver and gastrocnemius muscles the activity of ACP was increased to 113.28% and 126.23% at 10 h in comparison to control mice, respectively. This elevation might be due to increase in lysosomal disintegration. Alkaline phosphatase is an important membrane bound enzymes found in all body tissues. It mediates the transport of metabolites across the membrane and plays an important role in protein synthesis (Pilo *et al.*, 1972).

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