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Effect of Purified Paper Wasp *Ropalidia marginata* Venom Toxin Enzyme Activity in Blood Serum, Liver, and Gastrocnemius Muscle of Albino Mice

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Abstract

In the present, investigation effects of sub-lethal dose of purified paper wasp *Ropalidia marginata* venom toxins were evaluated on important metabolic enzymes *i.e.* ALP ACP, GPT, GOT, LDH, and AchE enzyme activity in serum, liver, and gastrocnemius muscles of albino mice. Alkaline phosphatase was found to be increased up to 119.9% at the 6th hr of the toxin injection in comparison to control. This elevation may be due to cytolysis. Maximum increase *i.e.*, 153.33% level of glutamate pyruvate transaminase (GPT) was found at 6 hrs of 40% of 24-h LD_{50} treatment while it was found to be 151.1% at 6 hrs of 24 hr 80% of LD_{50}, venom injection. A significant elevation was observed in LDH activity in serum, liver, and muscles, while the activity of AchE was decreased in serum, liver, and gastrocnemius muscles of albino mice after injecting the sub-lethal dose of *Ropalidia marginata* venom. This increase in the activity of LDH produces liver damage, massive disintegration and necrosis of hepatic cells. This elevation in LDH level led to a significant increase in the glucose catabolism and elevated oxidative stress in muscle and liver cells. It also displays insufficient oxygen supply and consequently leads to cell death. In experimental animals, venom toxin treatment decreased AchE level, and animals showed muscular paralysis. When mice were treated with 40% and 80% of 24-h LD_{50} of purified venom caused a significant ($p < 0.05$) elevation in the level of ACP, GOT, GPT, and LDH while the reduction in ALP and AChE level. Present study will be useful in the development of prototypes for study of pharmacological and therapeutic effects of various venom toxins. For this purpose structure activity relationship of enzyme and venom toxin, its due interaction to various metabolic enzymes and receptors must be explored.

Keywords

Ropalidia marginata, Serum, Liver and Rectus Abdominis, Gastrocnemius, Muscle, Atria and Ventricle Acid Phosphatase (ACP), Alkaline Phosphatase (ALP), Glutamate Pyruvate Transaminase (GPT) and Glutamate Oxaloacetate Transaminase (GOT), Lactic Dehydrogenase (LDH), Acetylcholinesterase (AchE)

1. Introduction

The wasps belong to the genus and order Hymenoptera. These are medium-sized insects, natural predators of lepidopteran larvae, and found in all parts of the world. Wasps play an important role in pollination, natural pest control, and biodiversity [1]. More than 30,000 species of hymenopteran wasps have been reported worldwide. Out of which, 22 recognized species belong to genus Vespa [2]. The social wasps build community nests by chewing wood to make a papery pulp. In groups as well as alone wasps are highly aggressive and attack very fast to protect their nesting sites. After feeling little disturbance, they quickly use stings that are more dangerous than those of bees [3]. Wasp venom after infliction causes severe pain, inflammation and inhibits metabolites including enzymes that lead to the failure of major metabolic pathways related to insect growth [1] [4]. If envenomation takes place by group of wasps toxic effects persist for longer period and victims face fatal consequences.

Social wasps mainly *Ropalidia marginata* (Hymenoptera: Vespidae) venom is a mixture of low molecular toxin peptides *i.e.*, phospholipases (phospholipase A2), acid phosphatase, proteases, hyaluronidase, metalloendopeptidase, Mastoparan-C (MP-C), scapin A, apamin, mast cell degranulating (MCD) peptide, polyamines, hornet in, bradykinin, AMPs which display diverse therapeutic potential. These show multiple biological activities *i.e.*, hemolytic and catalytic activity, and form pores in biological membranes [4]. Wasp's stings also contain a large amount (5%) of acetylcholine that causes heavy muscular pain. Wasp venom allergens induce specific reactions and evoke immune responses with more severe physiological changes [5]. Asian giant hornet (*Vespa mandarinia*) inflicts venom very quickly and causes human fatalities [6]. After infliction its venom cause massive inflammation, swelling, pain, and pathological effects in men and pets. Its toxins induce severe allergy and anaphylactic shock in victims. It obstructs respiration due to extensive swelling of the tracheal region [7]. If earlier treatment is not provided, it causes multiple organ failure that results in the death of the patient [8]. Wasp venoms also contain neurotoxic peptides and proteins mainly toxins and allergens [9].

Hymenopteran sting glands are the reservoirs of venom proteins/toxins. Wasp venom contains wide variety of substances such as toxins, enzymes, growth factor activators, and inhibitors [10]. These enzymes cause the disruption of cellu-

lar membranes and induce hypersensitive reactions, including life-threatening anaphylaxis. These target various bio-molecules mainly enzymes and impose cytotoxicity [11]. These are highly allergenic and trigger an IgE-induced immune response in susceptible individuals [12] [13]. Venom envenomation imposes severe pain, paralysis, allergic reactions, and biological effects on man and his pets [14]. To overcome the effects of venom toxins, early diagnosis, and venom immunotherapy is highly essential [15]. To tackle with the wasp venoms if victims show mild and moderate reactions to wasp stings apply a cold pack to wound site, wash the sting area with soap and water to remove venom, clean it with spirit or alcohol, and use hydrocortisone lotion over the skin surface. Medicated skin cream, baking soda and colloidal oatmeal rub over the stinger site. Vinegar is also used to lower down the alkalinity of wasp stings. Vinegar soaked ball sooth pain and reduce inflammation. Use anti-histamine drugs diphenhydramine and chlorpheniramine with pain killers to reduce inflammation and swelling.

Though, anti-venom administered neutralization takes 4 - 5 hrs in restoration of levels of bio-molecules [16]. In the present study, the sub-lethal dose of purified paper wasp *Ropalidia marginata* venom toxins was administered into albino mice, and enzyme activity ACP, ALP, GPT, GOT, LDH, AchE in blood serum, liver, and gastrocnemius muscle was investigated at various time intervals. Effects of purified bioactive peptides were also determined in gastrocnemius muscle, ventricle, atria, and liver at different time intervals.

2. Experimental

2.1. Collection of *Ropalidia marginata* Toxins

The living specimens of paper wasp *Ropalidia marginata* were collected from rural areas of the Gorakhpur district by using nylon net. The collected wasps were immobilized by quick freezing at −20˚C. Insects were dissected for taking out sting glands; its homogenate was prepared in phosphate buffer saline (50 mm, pH 6.9) with the help p of power homogenizer. The homogenate was centrifuged at 10,000 rpm at 4˚C for 10 minutes and the supernatant was used as crude venom.

2.2. Preparation of Homogenate

Ropalidia marginata sting 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 12,000 rpm in cold for 30 minutes and the supernatant was separated out. Total protein contents were estimated in the different supernatants according to Lowry's (1951) [17].

2.3. Purification of Venom Protein of *Ropalidia marginata*

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 known volume *i.e.*, 5 ml of toxin proteins solubilized in PBS was loaded in the column and the flow rate was maintained between 5 ml/minute by using a continuous buffer supply in a cold room. Elution of the venom proteins through a gel filtration column was done in a cold room at 4˚C at the flow rate of 5 ml/minute. A total of 121 regular fractions were collected at a fixed time interval using a Pharmacia fraction collector and the values of protein concentration in different eluted fractions will be plotted on the graph; absorbance in each fraction was determined at 280 nm using Shimadzu spectrophotometer (UV 2001 PC). Further, the absorbance of the same fractions was taken at 640 nm after protein estimation by Lowery (1951) [17].

2.4. Spectrophotometric Analysis of Proteins Eluted Fractions

The protein content eluted in each fraction was determined at a wavelength of 280 nm. A graph was plotted between absorption at 280 nm by using absorbance obtained in continuous fractions. Proteins/toxins eluted in the same faction numbers were determined at 640 nm by using the method of Lowry *et al.*, (1951) [17]. Values were also plotted for preparing the graph.

2.5. Molecular Weight Determination of Purified Venom Proteins

The range of molecular weight of different proteins/toxins in the purified paper wasp *Ropalidia marginata* toxins/proteins was determined by running the proteins of known molecular weight through the 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 the calibration curve range of molecular weight of different proteins in the purified *Ropalidia marginata* protein/toxins was determined.

2.6. Lyophilization of Eluted Venom Protein

The eluted fractions containing venom proteins were pooled and lyophilized to a desired concentration of venom toxins/proteins. Biological activity of the purified venom protein biological activity testing of *Ropalidia marginata* toxins was determined in albino mice serially known volumes of the purified toxins was injected intra-peritoneal.

2.7. Determination of Lethality of *Ropalidia marginata* Venom Toxins

The albino mice were injected subcutaneously with the purified venom toxins of different serial concentrations and LD_{50} was determined at intervals of the 24 hours. Deformities such as paralysis and neurotoxic effects were also recorded. Six albino mice injected with serial concentrations of the venom toxins to determine LD_{50}. 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 methods. The confidence limits were calculated at 95% probability levels. All bioassays were conducted by administering 40% and 80% of the LD_{50} of purified venom toxins.

2.8. Determination of Alkaline Phosphatase (ALP)

The Alkaline phosphatase level was determined according to the method of Andrech and Szeypiaske and modified by Bergmeyer [18]. For this purpose 500 mg treated termites were homogenized in 1 ml ice-cold PBS buffer and centrifuged at 15,000 ×g for 15 min. For measurement of alkaline phosphatase level, 0.10 ml of supernatant was added to 1.0 ml of alkaline buffer substrate and incubated for 30 minutes at 37˚C. Alkaline buffer substrate was prepared by addition of 375 mg glycine, 10 mg $MgCl_2 \cdot 6H_2O$ and 165 mg p-nitrophenyl phosphate sodium salt in 42 ml of 0.1 N NaOH. The reaction was stopped by adding an excess of alkali (5.0 ml of 0.02 N NaOH). The p-nitrophenol formed after the hydrolysis of p-nitrophenyl phosphate gave a yellow color with NaOH. Optical density was measured at 420 nm. A standard curve was prepared by using different concentrations of p-nitrophenol. Enzyme activity was expressed as μ moles of p-nitrophenol formed/30min/mg protein. Three replicates were set in each experiment and the data obtained was statistically analyzed by the ANOVA method.

2.9. Determination of Acid Phosphatase (ACP)

Acid phosphatase activity in termites was determined according to the method of Andrech and Szeypiaske and modified by Bergmeyer [18]. For the determination of acid phosphatase level, whole body extract of termites was prepared similarly as mentioned above. For this purpose, 0.1 ml of supernatant was added to 1.0 ml of acid buffer substrate solution (0.41 gm citric acid, 1.125 gm sodium citrate and 165 mg p-nitrophenyl phosphate sodium salt to 100 ml of double distilled water). Contents were mixed thoroughly and incubated for 30 minutes at 37˚C. To this tube 4.0 ml of 0.10 N NaOH was added to stop the reaction. A yellow color was developed which was measured at 420 nm. The standard curve was prepared by using different concentrations of p-nitrophenol. Enzyme activity was expressed as the amount of p-nitrophenol formed/30min/mg protein. Three replicates were set in each experiment and the data obtained was statistically analyzed by the ANOVA method.

2.10. Determination of Glutamic-Oxaolacetic Transaminase (GOT)

GOT activity was measured according to the method of Reitman and Frankel [19]. For this purpose, 500 mg treated termites were homogenized in 2 ml ice-cold PBS buffer and centrifuged at 15,000 rpm for 15 min. For estimation of GOT 0.10 ml of supernatant was taken and 0.50 ml of GOT substrate was added to it. GOT substrate was prepared by adding 0.292 gm of α-ketoglutaric acid and

26.6 gm of DL-aspartic acid into a 1.0-liter volumetric flask. Contents were mixed thoroughly and 1 N NaOH was added slowly to the above solution. pH of the solution was adjusted to 7.4 by using PBS buffer. The total volume of the solution was maintained at 1000 ml by adding buffer (13.97 gm K_2HPO_4 and 2.69 gm KH_2PO_4 in 1000 ml water). To this tube, 0.50 ml of 2 - 4 dinitrophenyl hydrazine solution (0.198 gm of 2,4-dinitrophenyl hydrazine was dissolved in 1 N HCl to make 1000 ml.) was added and kept standing for 15 minutes at room temperature. Then 5.0 ml of 0.4 N NaOH (1.6 gm NaOH dissolved in 100 ml distilled water) was added and mixed well. Now contents were left for 20 minutes at room temperature. Optical density was recorded at 505 nm by setting the blank with distilled water. The standard curve was prepared by using oxaloacetic acid as the standard. Enzyme activity was expressed in units of glutamate oxaloacetate transaminase/30min/mg protein.

2.11. Determination of Glutamic-Pyruvate Transaminase (GPT)

GPT activity in whole body extract of termites was measured according to the method of Reitman and Frankel [19]. For this purpose worker termites (500 mg) were treated and homogenized after 2 hrs in 2 ml ice-cold PBS buffer and centrifuged at 15,000 rpm for 15 min. 0.10 ml of centrifuged supernatant was added to 0.50 ml of GPT substrate. GPT substrate was prepared by dissolving 0.292 gm of α-ketoglutaric acid and 17.8 gm of DL alanine in a 1.0-liter volumetric flask. 1 N NaOH was slowly added to the above mixture. It was mixed well until all solids dissolved completely. The pH of the substrate was adjusted to 7.4 by adding a sufficient volume of buffer and the total volume was maintained at 1000 ml. The buffer was prepared by dissolving 13.97 gm K_2HPO_4 and 2.69 gm KH_2PO_4 in 1000 ml distilled water. In the above supernatant, 0.5 ml of GPT substrate and 0.50 ml of 2 - 4 dinitrophenyl hydrazine solution (0.198 gm of 2,4-dinitrophenyl hydrazine was dissolved in 1 N HCl to make 1000 ml) were added and kept for 15 minutes at room temperature. Now 5.0 ml of 0.4 N NaOH (1.6 gm NaOH dissolved in 100 ml distilled water) was added, mixed well and allowed to stand at room temperature for 20 minutes. The optical density was noted at 505 nm and the blank was set with water to make the background absorbance zero. The standard curve was prepared by using oxaloacetic acid as the standard. The enzyme activity was expressed in units of glutamic-pyruvate transaminase activity/mg protein. Three replicates were set for each test and the control and data obtained were statistically analyzed by the ANOVA method.

2.12. Determination of Acetyl Cholinesterase (AchE)

Acetylcholinesterase activity was determined according to the method of Ellman [20]. For this purpose, 500 mg treated termites were homogenized in ice-cold PBS buffer for 5 minutes in a glass-glass homogenizer. It was centrifuged at 15,000 rpm in cold to get the supernatant. For estimation of AchE level 0.050 ml of supernatant was mixed with (10 mm path length cuvette) 0.10 ml freshly pre-

pared acetyl choline thioiodide solution (5×10^{-4} M) and into it 0.05 ml DTNB (0.19818 gm/l) a chromogenic agent and 1.45 ml of PBS (pH 6.9) were added. The change in absorbance was recorded at 412 nm regularly for three minutes at 25˚C. Enzyme activity was expressed in μ moles "SH" hydrolyzed per minute per mg protein.

2.13. Statistical Analysis

The LD_{50} was determined after administration of increasing doses of for purified venom toxins in albino mice by using Probit analysis. Mean, standard deviation, standard error were determined according to Sokal and Rohlf [21].

3. Results

3.1. Solubilization of *Ropalidia marginata* Venom Toxins

Before extracting poisonous toxins, it was homogenized, and dissolved in different solution buffers, *i.e.*, Triton was isolated by homogenizing Triton X 100 (0.01%), Tris EDTA (0.1 mM) PBS buffer 78%, TCA 5%, and absolute alcohol, Triton X 100 (0.1%) proved to be good for solubilization of paper wasp *Ropalidia marginata* venoms because it showed 82.4% dissolution than any other solubilizing buffer used. Higher protein solubility was obtained in the supernatant than in the residue, except in TCA (Figure 1). Solubility in PBS buffer is about 78% (Figure 1).

3.2. Purification

For isolation and purification sting glands of *Ropalidia marginata* were taken out, and homogenized in 5 ml of PBS (pH 6.9) using a glass-glass homogenizer with 5 ml of solubilizing buffer. The homogenate was centrifuged in the cold at 4˚C for 30 minutes at 15,000 rpm and the supernatant was carefully separated. It

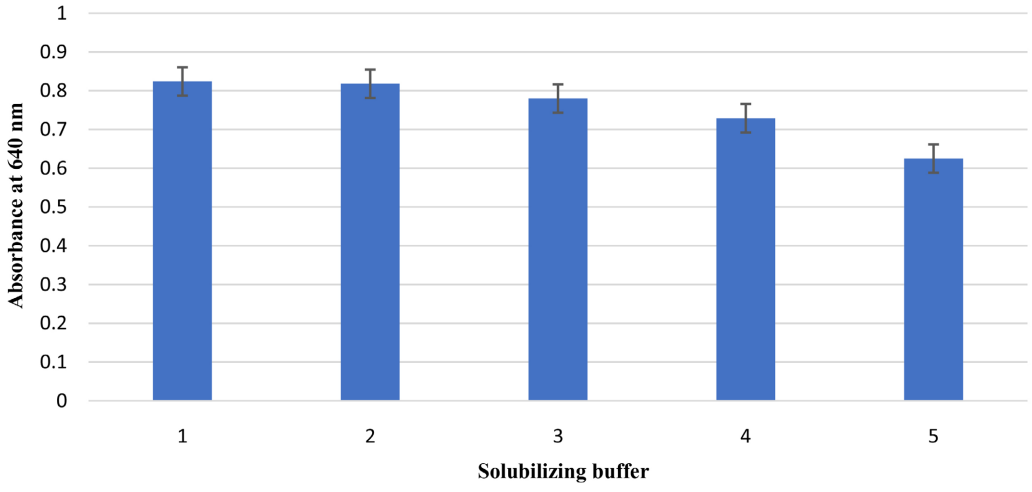

Figure 1. Solubilization of *Ropalidia marginata* in different buffers. Absorbance of solubilized protein was taken at 640 nm. Solubilizing buffers on X-axis are (1) Triton X 100 (0.01%), (2) Tris + EDTA (0.1 Mm) (3) PBS buffer (4) TCA 5% and (5) Absolute alcohol.

was loaded onto a Sepharose CL-6B 200 column to separate the venom toxins. The venom toxin homogenate elution pattern showed five major peaks at 280 nm. Immediately after the void fraction, there are three peaks at 37 - 42, 46 - 51, and 64 - 71, while the fourth and fifth peaks were present at 81 - 97 (Figure 2). In addition, the concentration of *Ropalidia marginata* toxin was determined in each test tube using the method of Lowry (1951). Again, two similar protein peaks were resolved at 640 nm (Figure 3). The first peak was a large peak between 46 and 56, while the second peak was large and located between fractions numbers 61 and 67 (Figure 3). Both peaks were eluted with PBS buffer (pH 6.9). The total yield of poisonous toxins in the eluted fractions was 76.8%.

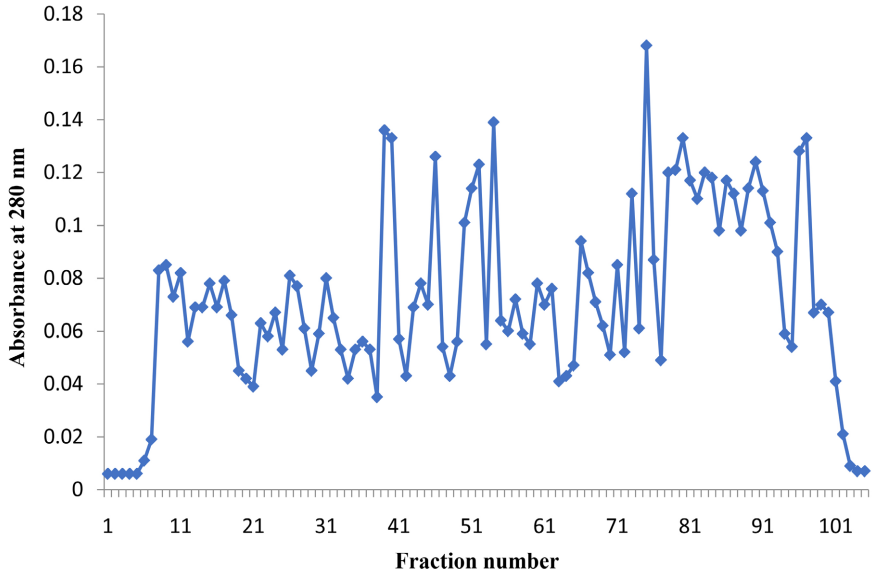

Figure 2. Elution pattern of PBS extractable proteins of *Ropalidia marginata* wasp chromatographed on a Sepharose CL-6B 200 column. Absorbance was taken at 280 nm.

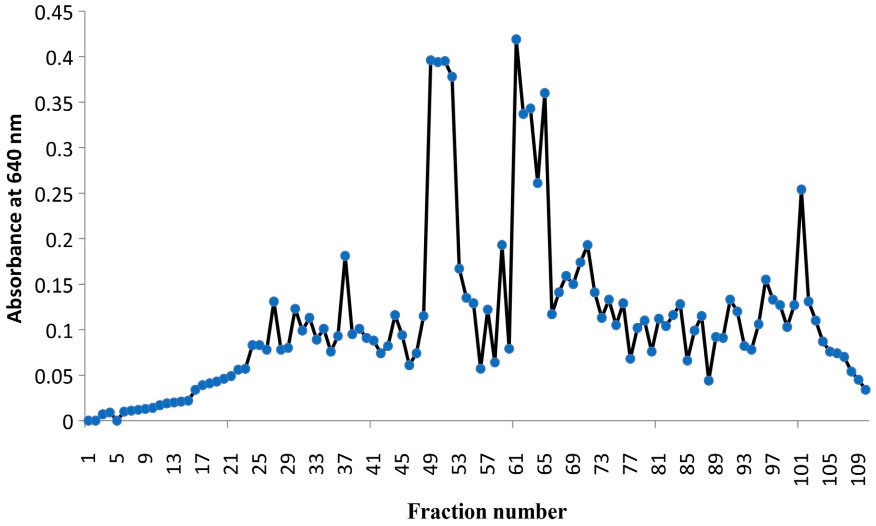

Figure 3. Elution pattern of PBS extractable proteins of *Ropalidia marginata* wasp chromatographed on a Sepharose CL-6B 200 column. Absorbance was taken at 640 nm.

3.3. Molecular Weight Determination of Wasp Venom Toxins

The molecular weight of *Ropalidia marginata* venom toxins/proteins was determined by Sepharose CL-6B 200 gel column chromatography using standard marker proteins of known molecular weight (Figure 3). The calibration curve shows that the molecular weight of the purified venom proteins is 12.6 - 63 kDa (Figure 4).

3.4. Venom Fractions

The eluted venom protein fractions were pooled and lyophilized. The toxicity of purified wasp venom toxins from *Ropalidia marginata* toxin was determined in various tissues of albino mice (*Mus musculus*). Wasp venom proteins obtained by lyophilization caused heavy toxicity in albino mice. The LD_{50} of *Ropalidia marginata* venom protein was found to be 20.6 ± 0.094 mg/kg body weight in albino mice.

Effects of purified *Ropalidia marginata* venom on various bio-molecules in the blood serum of albino mice were evaluated. Changes in the concentration of certain macromolecules, *i.e.*, proteins, free amino acids, uric acid, cholesterol, pyruvic acid, total lipids, and glucose were measured after an intraperitoneal 24-hour LD_{50} injection of 40% and 80% purified *Ropalidia marginata* venom toxins.

The toxic effect of the purified venom toxins of *Ropalidia marginata* was observed in albino mice on serum, liver, and gastrocnemius muscle enzyme activity of alkaline phosphatase and acid phosphatase. The albino mice were treated with 40% and 80% of 24 h LD_{50} of purified wasp toxins and alterations in enzyme activity were measured after 2, 4, 6, 8, and 10 h of treatment. Wasp venom caused a 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 (Table 1 and Table 2).

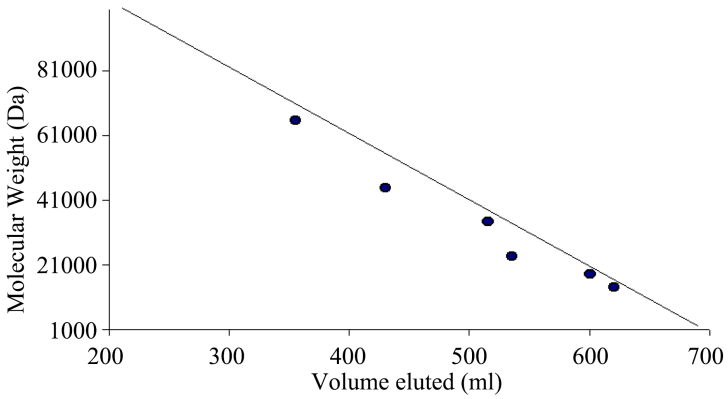

Figure 4. Standard proteins chromatographed on Sepharose CL-6B 200 column for determining the molecular weights peptides isolated from *Ropalidia marginata.* Proteins used were bovine albumin mol. wt. 66,000, egg albumin mol. wt. 45,000, pepsin mol. wt. 34,700, trypsinogen mol. wt. 24,000, beta lactoglobulin mol. Wt. 18,400 and lysozyme mol.

Table 1. *In vivo* effect of 40% of 24-h LD_{50} purified *Roplidia marginata* venom toxins on different enzymes *i.e.*, acid phosphatase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase, and acetylcholinesterase in mice serum.

Enzymes	Time in hrs.					
	0	2	4	6	8	10
ACP	0.31 ± 0.057 (100)	0.385 ± 0.0577 (124)	0.415 ± 0.0288 (132.2)	0.44 ± 0.011 (141.9)	0.36 ± 0.057 (116.1)	0.33 ± 0.190 (106.45)
ALP	2.11 ± 0.0577 (100)	2.41 ± 0.0288 (114.2)	2.44 ± 0.0288 (115.6)	2.53 ± 0.0288 (119.9)	2.44 ± 0.0288 (115.6)	2.49 ± 0.0288 (118.0)
GPT	0.045 ± 0.0288 (100)	0.055 ± 0.0288 (122.22)	0.068 ± 0.0288 (151.1)	0.069 ± 0.0288 (153.33)	0.0645 ± 0.0288 (143.33)	0.064 ± 0.0288 (142.22)
GOT	0.38 ± 0.0288 (100)	0.42 ± 0.0288 (110.5)	0.47 ± 0.0288 (123.68)	0.525 ± 0.0288 (138.1)	0.516 ± 0.0288 (135.7)	0.51 ± 0.0288 (134.2)
LDH	7.75 ± 0.0288 (100)	8.16 ± 0.0288 (105.29)	8.69 ± 0.0288 (121.12)	9.31 ± 0.0288 (120.12)	8.9 ± 0.0288 (114.83)	8.68 ± 0.0288 (111.07)
ACHE	0.038 ± 0.0577 (100)	0.0345 ± 0.0577 (89.61)	0.0305 ± 0.0288 (79.22)	0.028 ± 0.0288 (72.72)	0.0305 ± 0.0288 (79.22)	0.0325 ± 0.0288 (84.41)

Values are mean ± SE of three replicates. Values in parentheses indicate per cent enzyme activity with respect to control taken as 100%. Significant ($p < 0.05$, Student t-test). Acid phosphatase (ACP) and Alkaline phosphatase (ALP): μ moles of p-nitrophenol formed/30minute/mg protein. Glutamate pyruvate transaminases (GPT): Units of Glutamate pyruvate transaminase activity/hour/mg protein. Glutamate oxaloacetate transaminase (GOT): Units of Glutamate oxaloacetate transaminase activity/hour/mg protein. Lactic dehydrogense (LDH): μ moles of pyruvate reduced/45minute/mg protein. Acetylcholinesterase (AchE): μ moles "SH" hydrolysed/minute/mg protein.

Table 2. *In vivo* effect of 80% of 24-h LD_{50} purified *Roplidia marginata* venom toxins on different enzymes *i.e.*, acid phosphatase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase, and acetylcholinesterase in mice serum.

Enzymes	Time in hrs.					
	0	2	4	6	8	10
ACP	0.315 ± 0.0288 (100)	0.38 ± 0.0577 (122.58)	0.415 ± 0.0288 (132.2)	0.455 ± 0.0288 (145.1)	0.375 ± 0.0288 (119.35)	0.345 ± 0.0288 (138.70)

Continued

ALP	2.21 ± 0.0577 (100)	2.41 ± 0.0288 (109.04)	2.45 ± 0.0288 (110.8)	2.53 ± 0.0288 (114.47)	2.46 ± 0.0288 (111.31)	2.44 ± 0.0288 (110.40)
GPT	0.045 ± 0.0288 (100)	0.0565 ± 0.0288 (124.44)	0.066 ± 0.0288 (146.66)	0.068 ± 0.0288 (151.11)	0.066 ± 0.0288 (146.66)	0.067 ± 0.0288 (148.8)
GOT	0.38 ± 0.0288 (100)	0.435 ± 0.0288 (113.15)	0.46 ± 0.0288 (121.05)	0.525 ± 0.0288 (138.15)	0.525 ± 0.0288 (138.15)	0.51 ± 0.0288 (134.21)
LDH	7.73 ± 0.0288 (100)	8.245 ± 0.0288 (106.6)	8.69 ± 0.0288 (112.41)	9.27 ± 0.0288 (119.92)	8.93 ± 0.0288 (115.5)	8.66 ± 0.0288 (112.03)
ACHE	0.038 ± 0.0577 (100)	0.0345 ± 0.0577 (90)	0.030 ± 0.0288 (78)	0.028 ± 0.0288 (73.6)	0.030 ± 0.0288 (78.9)	0.032 ± 0.0288 (84.24)

Values are mean ± SE of three replicates. Values in parentheses indicate per cent enzyme activity with respect to control taken as 100%. Significant ($p < 0.05$, Student t-test). Acid phosphatase (ACP) and Alkaline phosphatase (ALP): μ moles of p-nitrophenol formed/30minute/mg protein. Glutamate pyruvate transaminases (GPT): Units of Glutamate pyruvate transaminase activity/hour/mg protein. Glutamate oxaloacetate transaminase (GOT): Units of Glutamate oxaloacetate transaminase activity/hour/mg protein. Lactic dehydrogense (LDH): μ moles of pyruvate reduced/45minute/mg protein. Acetylcholinesterase (AchE): μ moles "SH" hydrolysed/minute/mg protein.

3.5. Effects of Purified *Ropalidia marginata* Venom Toxin on Certain Blood Serum Enzymes

Changes in the level of various serum enzymes *i.e.*, acid phosphatase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactate dehydrogenase, acetylcholinesterase were observed in albino mice after providing an injection of 40% and 80% of 24-h LD_{50} at different time duration (2 hr, 4 hr, 6 hr, 8 hr, 10 hr) in control groups of mice were injected only with PBS buffer (6.9) (Table 1 and Table 2 and Figures 5-10).

Acid phosphatase level (ACP) was found to be increased significantly ($p < 0.05$) up to 145.1% and 109.52% at 6 and 10 hrs of 24-h LD_{50} of *Ropalidia marginata* venom injection with respect to control. Further, LD_{50} recovered 83% at the 10 hrs in comparison to control respectively (Table 1 and Table 2 and Figure 5).

Similarly, a significant ($p < 0.05$) decrease in alkaline phosphatase (ALP) level was obtained at 29% and 37% at 6 hrs of 40% and 80% of 24-h LD_{50} of *Ropalidia marginata* treatment with respect to control. Further, it was found to be 81% and 73% at 10 hrs (Table 1 and Table 2 and Figure 6).

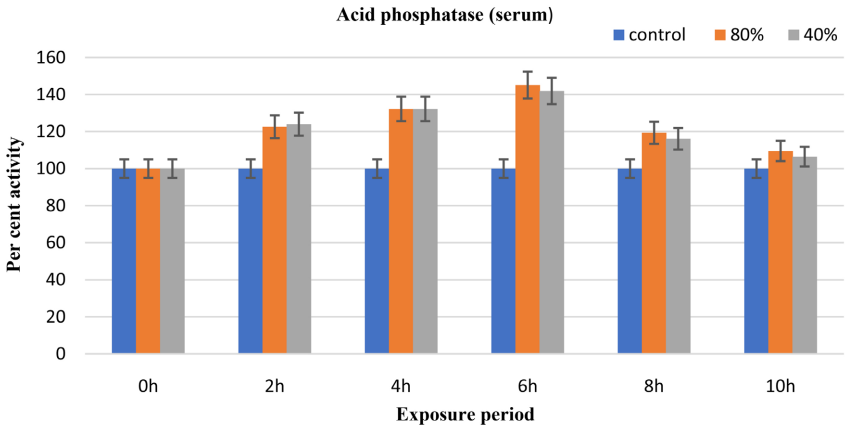

Figure 5. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of serum acid phosphatase in albino mice.

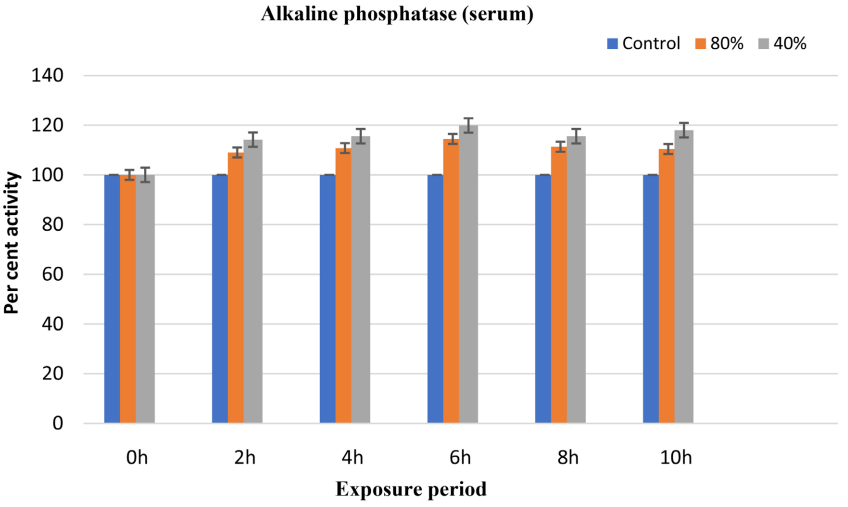

Figure 6. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on *activity* of serum alkaline phosphatase in albino mice.

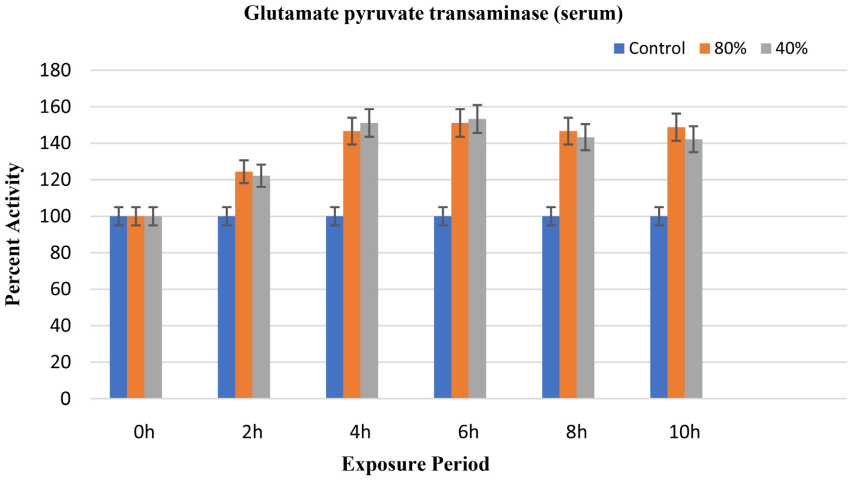

Figure 7. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of serum glutamate pyruvate transaminase in albino mice.

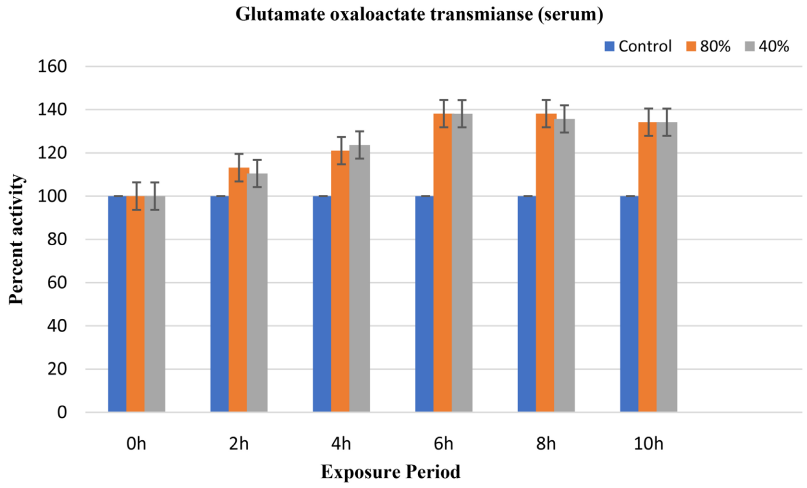

Figure 8. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of serum glutamate oxaloactate transaminase in albino mice.

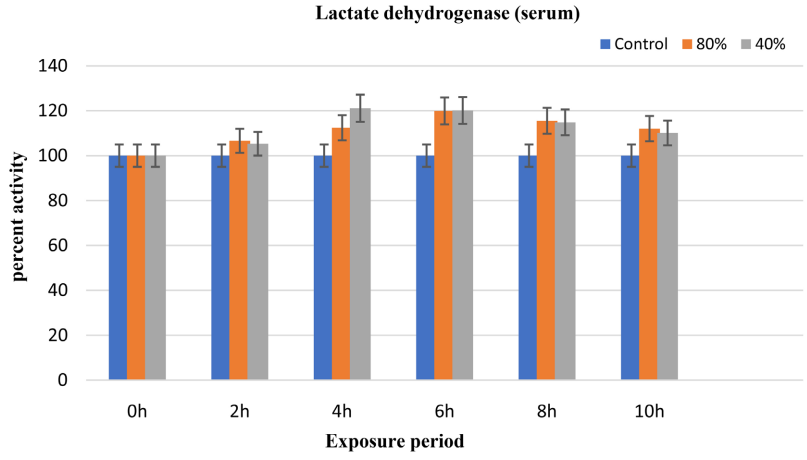

Figure 9. *In vivo* effect of 40% and 80% of 24-h LD_{50} OF purified venom toxins of *Ropalidia marginata* on activity of serum lactate dehydrogenase in albino mice.

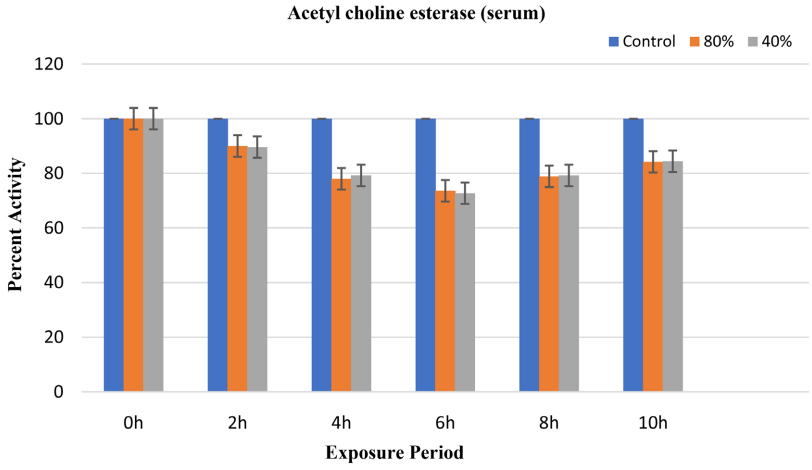

Figure 10. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of serum acetyl choline esterase in albino mice.

Maximum increase *i.e.*, 153.33% level of glutamate pyruvate transaminase (GPT) was found at 6 hrs of 40% of 24-h LD_{50} treatment while it was found to be 151.1% at 6 hrs of 24 hr 80% of LD_{50}, venom injection. Further, it recovered 142.22% and 148.88% at 10 hrs. of 40% and 80% of 24 hr LD_{50} with respect to control (Table 1 and Table 2 and Figure 7).

Similarly glutamate oxaloacetate transaminase (GOT) level significantly ($p < 0.05$) increased up to 134.21% and 138.15% at 10 hr and 8 hrs of 40% and 80% of 24 h LD_{50} treatment respectively (Table 1 and Table 2 and Figure 8); A significant ($p < 0.05$) increased in LDH obtained was 120.12% and 119.92% at 6 hrs of 40% and 80% of 24-h LD_{50} of *Ropalidia marginata* venom treatment with respect to control (Table 1 and Table 2 and Figure 8).

At similar doses of venom toxins, LDH level was found to be elevated significantly ($p < 0.05$) up to 120.12% and 119.92% at 6 hrs of 40% and 80% of 24-h LD_{50} of purified *Ropalidia marginata* venom injection with respect to control. Later on, it recovered up to 112.03% and 111.07% at 10 hrs in comparison to the control (Table 1 and Table 2 and Figure 9).

Contrary to this, the Ache level decreased significantly ($p < 0.05$) up to 80% and 40% at 6 hrs of treatment with 40% and 80% of 24-h LD50 of purified *Ropalidia marginata*venom respectively. Further, it was found 84.41% and 84.21% at 10 hrs after the treatment with the same doses of the venom with respect to control (Table 1 and Table 2 and Figure 10).

3.6. Effect of Purified *Ropalidia marginata* Venom Toxins on Certain Enzymes in the Liver

This section deal with the effect of purified *Ropalidia marginata* venom toxins on different enzymes in the liver of albino mice. When mice were treated with 40% and 80% of 24-h LD50 of purified venom caused a significant ($p < 0.05$) elevation in the level of ACP, GOT, GPT, and LDH while the reduction in ALP and AChE (Table 3 and Table 4 and Figures 11-16).

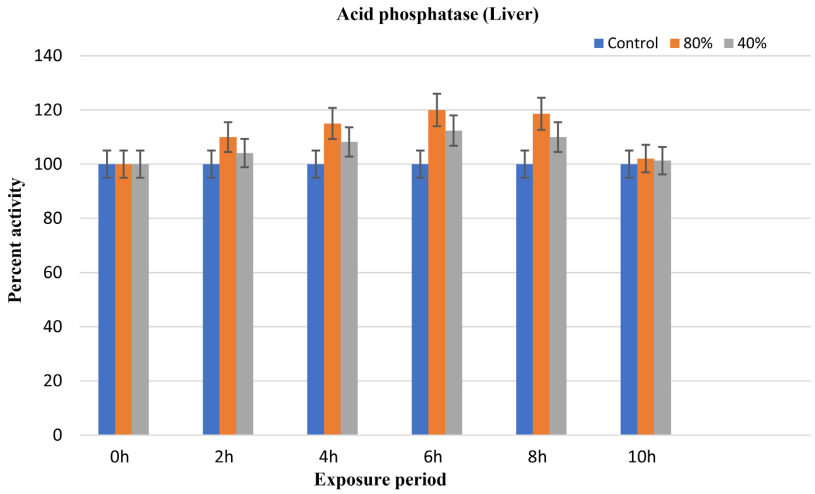

Figure 11. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of acid phosphatase in albino mice.

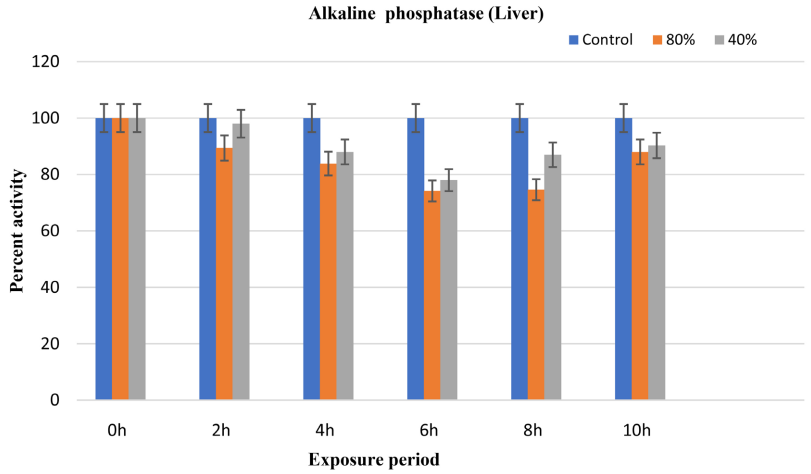

Figure 12. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of liver alkaline phosphatase in albino mice.

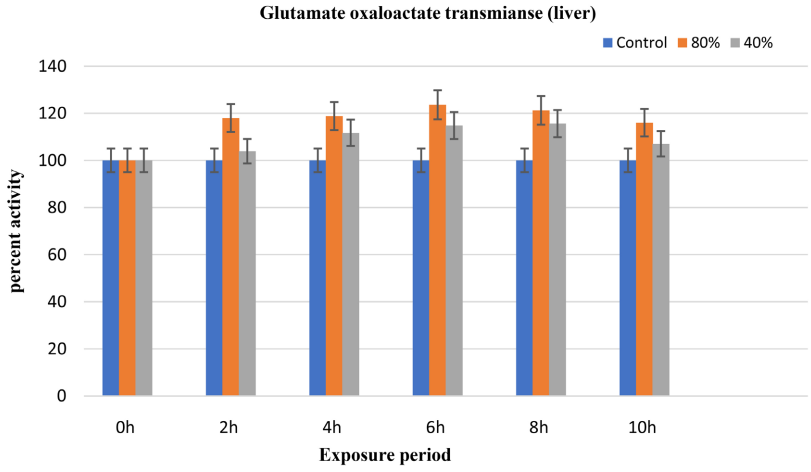

Figure 13. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of liver glutamate oxaloacetate transaminase in albino mice.

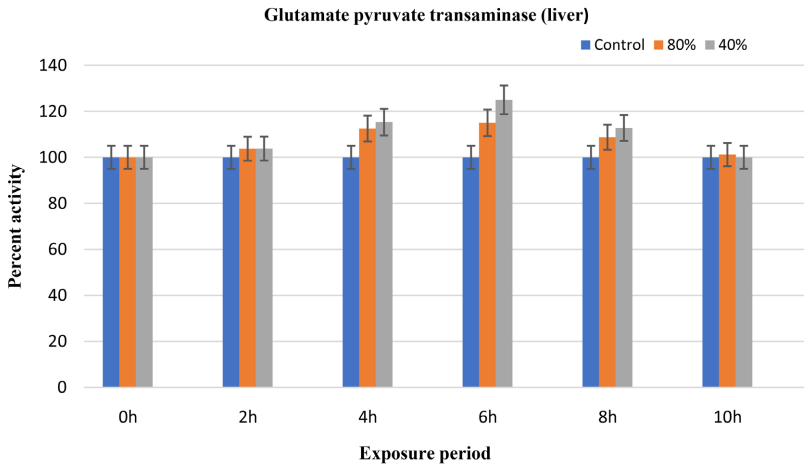

Figure 14. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* activity of liver glutamate pyruvate transaminase in albino mice.

Table 3. *In vivo* effect of 40% of 24-h LD_{50} purified *Roplidia marginata* venom toxins on different enzymes *i.e.*, acid phosphatase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase, and acetylcholinesterase in the liver of albino mice.

Enzymes	Time in hrs					
	0	2	4	6	8	10
ACP	1.45 ± 0.0816 (100)	1.51 ± 0.0816 (104.1)	1.57 ± 0.0816 (108.2)	1.63 ± 0.0816 (112.4)	1.61 ± 0.0816 (110)	1.47 ± 0.0816 (101.3)
ALP	2.17 ± 0.0816 (100)	2.14 ± 0.0816 (98)	1.93 ± 0.0816 (88)	1.71 ± 0.0816 (78)	1.90 ± 0.0816 (87)	1.96 ± 0.0816 (90.3)
GPT	0.78 ± 0.0816 (100)	0.81 ± 0.0816 (103.8)	0.90 ± 0.0816 (115.3)	0.98 ± 0.0816 (125)	0.88 ± 0.0816 (112.8)	0.78 ± 0.0816 (100)
GOT	1.28 ± 0.0816 (100)	1.33 ± 0.0816 (103.9)	1.43 ± 0.0816 (111.7)	1.47 ± 0.0816 (114.8)	1.48 ± 0.0816 (115.6)	1.37 ± 0.0816 (107.03)
LDH	1.78 ± 0.0816 (100)	1.95 ± 0.0816 (109.5)	2.01 ± 0.0816 (112.9)	2.21 ± 0.0816 (124.15)	1.95 ± 0.0816 (109.5)	1.82 ± 0.0816 (102.2)
ACHE	0.051 ± 0.0816 (100)	0.043 ± 0.0816 (84.31)	0.041 ± 0.0816 (80.39)	0.040 ± 0.0816 (78.43)	0.044 ± 0.0816 (86.27)	0.049 ± 0.0816 (96.07)

Values are mean ± SE of three replicates. Values in parentheses indicate per cent enzyme activity with respect to control taken as 100%. Significant ($p < 0.05$, Student t-test). Acid phosphatase (ACP) and Alkaline phosphatase (ALP): μ moles of p-nitrophenol formed/30minute/mg protein. Glutamate pyruvate transaminases (GPT): Units of Glutamate pyruvate transaminase activity/hour/mg protein. Glutamate oxaloacetate transaminase (GOT): Units of Glutamate oxaloacetate transaminase activity/hour/mg protein. Lactic dehydrogense (LDH): μ moles of pyruvate reduced/45minute/mg protein. Acetylcholinesterase (AchE): μ moles "SH" hydrolysed/minute/mg protein.

Table 4. *In vivo* effect of 80% of 24-h LD_{50} purified *Roplidia marginata* Venom toxins on different enzymes *i.e.*, Acid phosphatase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase, acetylcholinesterase in the liver of albino mice.

Enzymes	Time in hrs.					
	0	2	4	6	8	10
ACP	1.45 ± 0.0816 (100)	1.61 ± 0.0816 (110)	1.67 ± 0.0816 (115)	1.74 ± 0.0816 (120)	1.72 ± 0.0816 (118.6)	1.46 ± 0.0816 (102.6)

Continued

ALP	2.17 ± 0.0816 (100)	1.94 ± 0.0816 (89.40)	1.82 ± 0.0816 (83.87)	1.61 ± 0.0816 (74.19)	1.62 ± 0.0816 (74.60)	1.91 ± 0.0816 (88)
GPT	0.080 ± 0.0816 (100)	0.83 ± 0.0816 (103.75)	0.90 ± 0.0816 (112.5)	0.92 ± 0.0816 (115)	0.87 ± 0.0816 (108.75)	0.81 ± 0.0816 (101.2)
GOT	1.27 ± 0.0816 (100)	1.42 ± 0.0816 (118)	1.51 ± 0.0816 (118.8)	1.57 ± 0.0816 (123.6)	1.54 ± 0.0816 (121.2)	1.48 ± 0.0816 (116)
LDH	1.78 ± 0.0816 (100)	1.98 ± 0.0816 (111.2)	2.11 ± 0.0816 (118.5)	2.57 ± 0.0816 (144)	1.98 ± 0.0816 (111.2)	1.78 ± 0.0816 (100)
ACHE	0.047 ± 0.0816 (100)	0.041 ± 0.0816 (87)	0.041 ± 0.0816 (87)	0.037 ± 0.0816 (78)	0.040 ± 0.0816 (85)	0.042 ± 0.0816 (89)

Values are mean ± SE of three replicates. Values in parentheses indicate per cent enzyme activity with respect to control taken as 100%. Significant ($p < 0.05$, Student t-test). Acid phosphatase (ACP) and Alkaline phosphatase (ALP): μ moles of p-nitrophenol formed/30minute/mg protein. Glutamate pyruvate transaminases (GPT): Units of Glutamate pyruvate transaminase activity/hour/mg protein. Glutamate oxaloacetate transaminase (GOT): Units of Glutamate oxaloacetate transaminase activity/hour/mg protein. Lactic dehydrogense (LDH): μ moles of pyruvate reduced/45minute/mg protein. Acetylcholinesterase (AchE): μ moles "SH" hydrolysed/minute/mg protein.

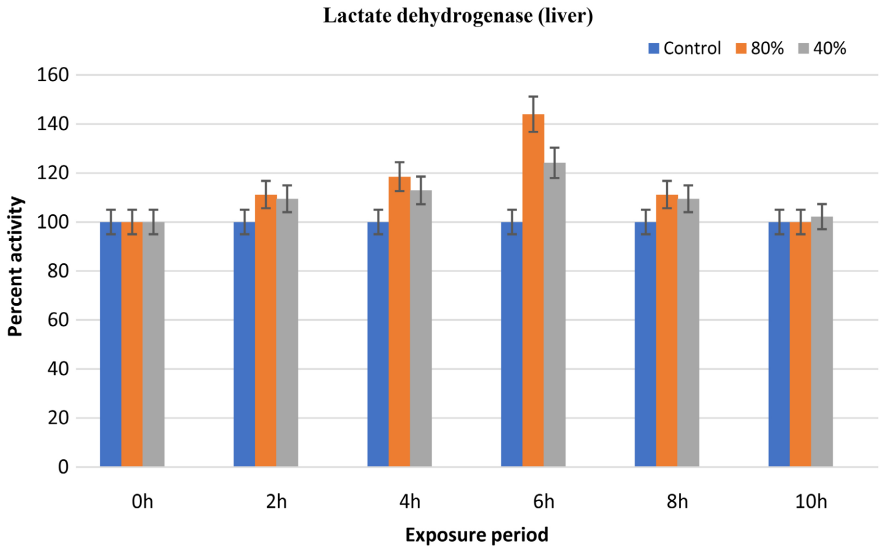

Figure 15. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* activity of liver lactate dehydrogenase in albino mice.

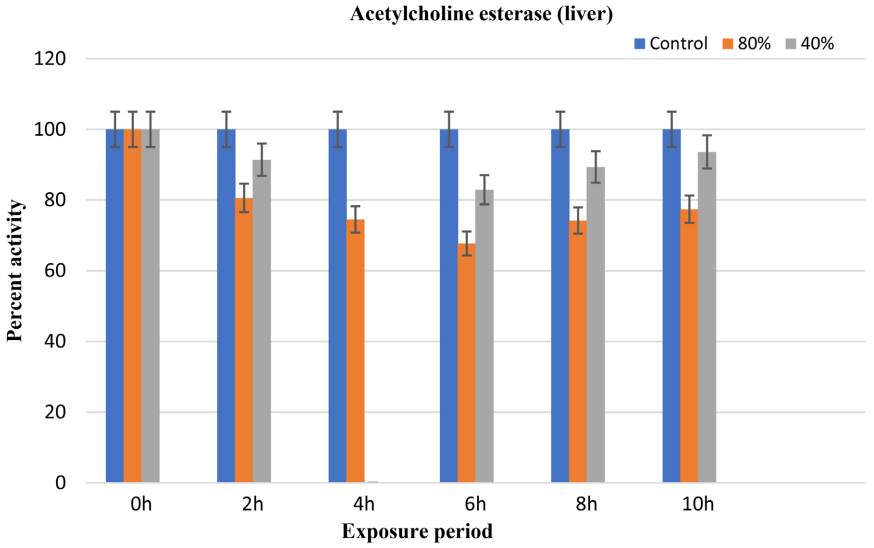

Figure 16. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* activity of liver acetyl choline esterase in albino mice.

ACP was found to be increased up to 112.4% and 120% at 6 hrs of treatment with 40% and 80% of 24-h LD_{50} of purified venom injection. Later on, at 10 hrs it was recovered to 102.06% and 101.3% with respect to control (**Table 3** and **Table 4** and **Figure 11**).

While ALP level was decreased significantly ($p < 0.05$) up to 78% and 74.19% at 6 hrs of treatment with 40% and 80% of 24-h LD_{50} of purified venom toxins. Further, it was recovered up to 90.3% and 88% at 10 hrs with respect to control (**Table 3** and **Table 4** and **Figure 12**).

A significant ($p < 0.05$) increase in GPT observed was 115% and 125% at 6 hrs of 40% and 80% of 24-h LD_{50} of venom injection with respect to control. Further, it recovered 100% and 101.2% at 10 hrs (**Table 3** and **Table 4** and **Figure 13**).

Similarly, GOT level was found to be increased significantly ($p < 0.05$) up to 123.6% at 6 hr 80% of 24-h LD_{50} and 114.8% at 6 hrs of 40% of 24-h LD_{50} of purified *Ropalidia marginata* venom treatment. Later on, it was recovered up to 116% and 107.03% at 10 hrs of treatment in comparison to control respectively (**Table 3** and **Table 4** and **Figure 14**).

At similar doses of venom toxins, LDH level was found to be elevated significantly ($p < 0.05$) up to 124.15% and 144% at 6 hrs of 40% and 80% of 24-h LD_{50} of purified *Ropalidia marginata* venom injection with respect to control. Later on, it recovered up to 102.2% and 100% at 10 hrs. In comparison to the control (**Table 3** and **Table 4** and **Figure 15**).

More specifically, Ache level was found to be decreased significantly ($p < 0.05$) up to 78.43% and 78% at 6 hrs of treatment with 40% and 80% of 24-h LD_{50} of purified *Ropalidia marginata* venom in comparison to control respectively. Later on, it improved up to 96.07% and 89% at 10 hrs with respect to control (**Table 3** and **Table 4** and **Figure 16**).

3.7. Effect of Purified *Ropalidia marginata* Venom Toxins on Certain Enzymes in Gastrocnemius Muscle of Albino Mice

In this section, the effect of 40% and 80% of 24-h LD_{50} purified *Ropalidia marginata*venom protein on different enzymes in mice gastrocnemius muscles of albino mice. After above treatment of albino mice level of various enzymes such as ACP, ALP, GOT, GPT, LDH, Ache was measured in gastrocnemius muscles at 2, 4, 6, 8, and 10 hrs of treatment. Mice injected only with buffer were considered as control, following deviation in enzymes level found in gastrocnemius muscle of albino mice (Table 5 and Figures 17-22).

Table 5. *In vivo* effect of 40% of 24-h LD_{50} purified *Roplidia marginata venom* toxins on different enzymes *i.e.*, acid phosphatase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase, and acetylcholinesterase in gastrocnemius muscle of albino serum.

Enzymes	Time in hrs.					
	0	2	4	6	8	10
ACP	0.86 ± 0.0816 (100)	1.04 ± 0.0816 (120)	1.11 ± 0.0816 (129)	1.2 ± 0.0816 (139)	1.15 ± 0.0816 (133)	1.03 ± 0.0816 (119)
ALP	0.75 ± 0.0816 (100)	0.66 ± 0.0816 (88)	0.63 ± 0.0816 (84)	0.56 ± 0.0816 (74)	0.57 ± 0.0816 (76)	0.62 ± 0.0816 (82.6)
GPT	1.57 ± 0.0816 (100)	1.65 ± 0.0816 (105)	1.74 ± 0.0816 (110)	1.76 ± 0.0214 (112)	1.73 ± 0.024 (110)	1.65 ± 0.816 (110)
GOT	0.37 ± 0.0124 (100)	0.45 ± 0.0124 (121)	0.48 ± 0.0816 (129)	0.50 ± 0.0816 (135)	0.43 ± 0.0124 (116)	0.41 ± 0.0816 (110)
LDH	90.28 ± 0.0816 (100)	91.45 ± 0.179 (101)	91.87 ± 0.0816 (101.7)	92.19 ± 0.0816 (102.9)	92.31 ± 0.0816 (102.2)	91.77 ± 0.0816 (101.6)
ACHE	0.0047 ± 0.0816 (100)	0.043 ± 0.0816 (91.4)	0.041 ± 0.0816 (87.23)	0.039 ± 0.0816 (82.9)	0.042 ± 0.0816 (89.36)	0.044 ± 0.0816 (93.61)

Values are mean ± SE of three replicates. Values in parentheses indicate per cent enzyme activity with respect to control taken as 100%. Significant ($p < 0.05$, Student t-test). Acid phosphatase (ACP) and Alkaline phosphatase (ALP): μ moles of p-nitrophenol formed/30minute/mg protein. Glutamate pyruvate transaminases (GPT): Units of Glutamate pyruvate transaminase activity/hour/mg protein. Glutamate oxaloacetate transaminase (GOT): Units of Glutamate oxaloacetate transaminase activity/hour/mg protein. Lactic dehydrogense (LDH): μ moles of pyruvate reduced/45minute/mg protein. Acetylcholinesterase (AchE): μ moles "SH" hydrolysed/minute/mg protein.

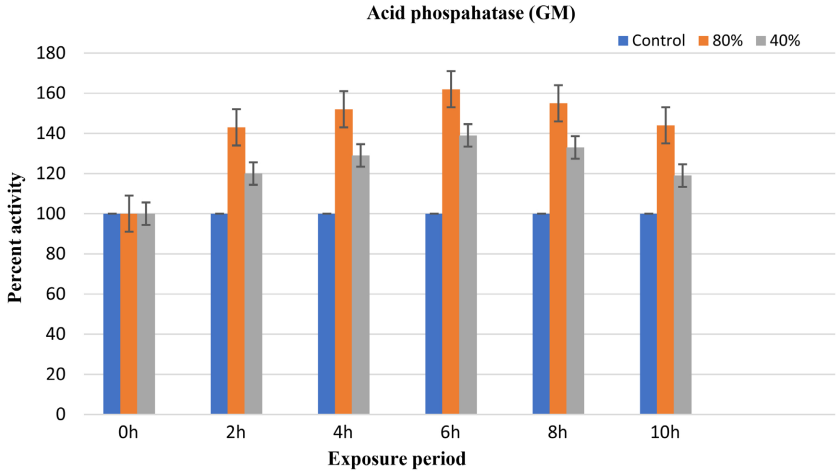

Figure 17. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of gastrocnemius muscle acid phosphatase in albino mice.

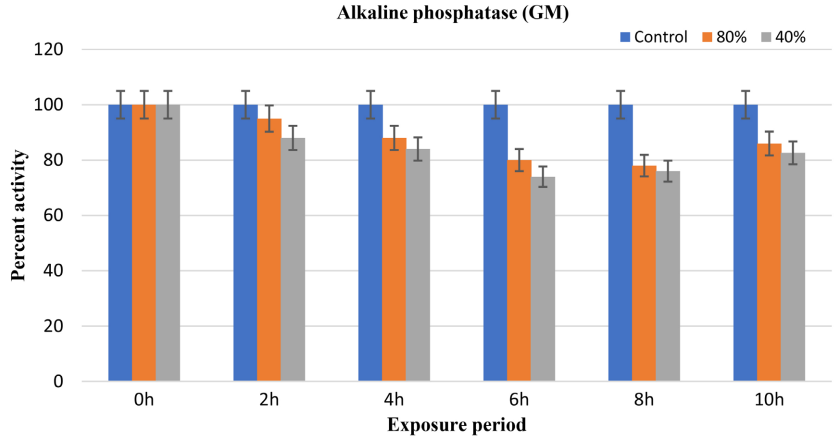

Figure 18. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of gastrocnemius muscle alkaline phosphatase in albino mice.

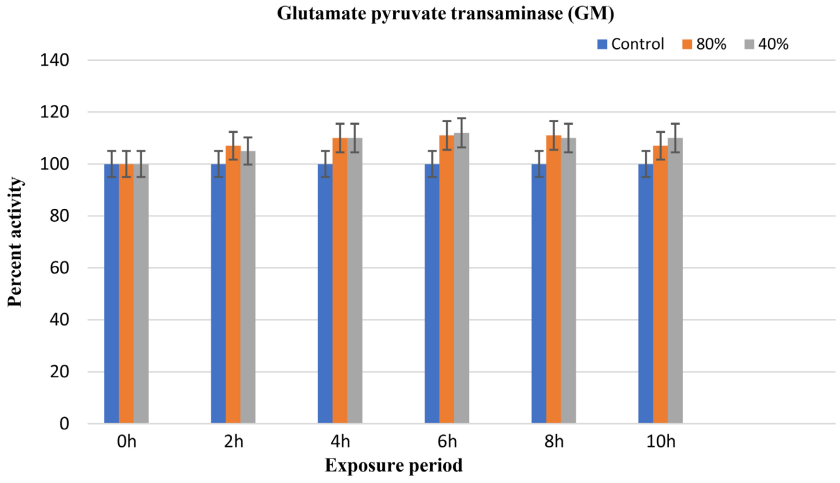

Figure 19. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of gastrocnemius muscle glutamate pyruvate transaminase in albino mice.

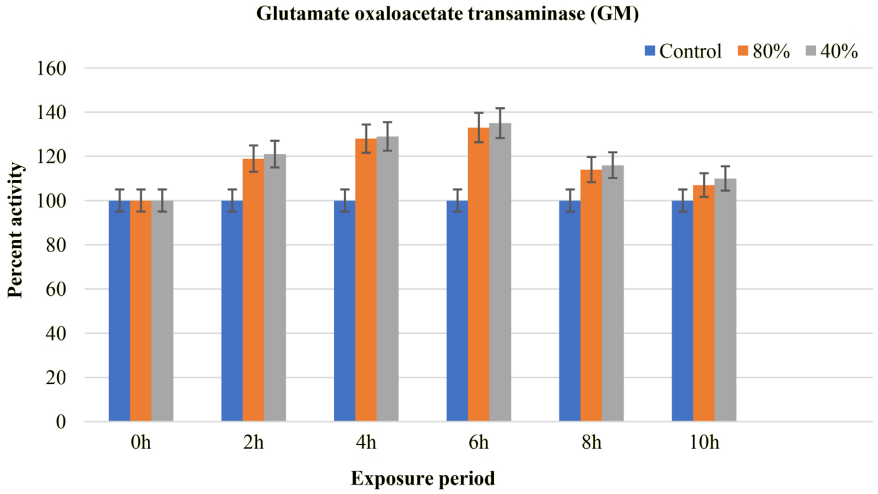

Figure 20. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of gastrocnemius muscle glutamate oxaloacetate transaminase in albino mice.

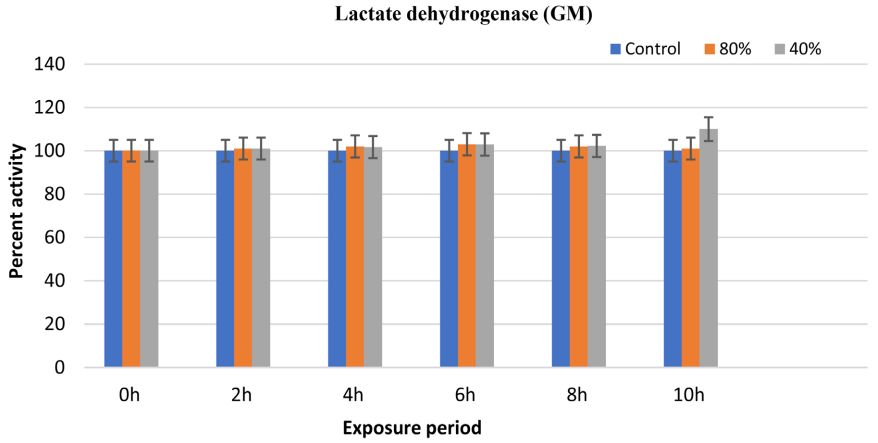

Figure 21. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of gastrocnemius muscle lactate dehydrogenase in albino mice.

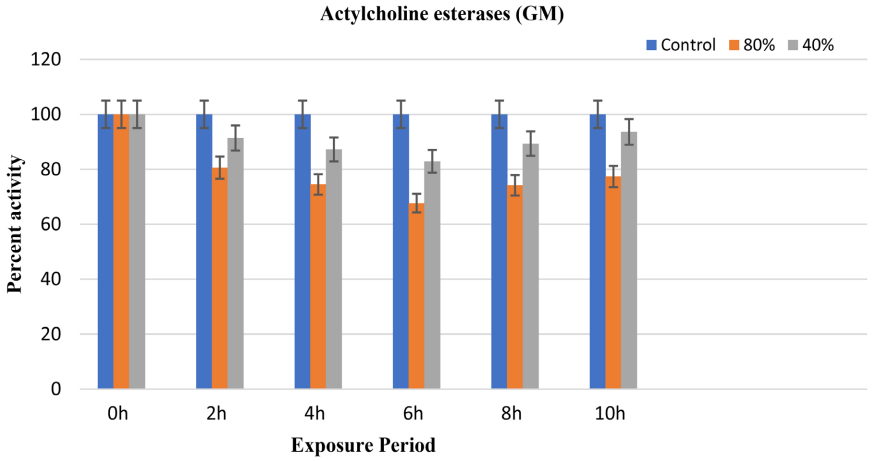

Figure 22. *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of *Ropalidia marginata* on activity of gastrocnemius muscle acetyl choline esterase in albino mice.

ACP level was increased significantly ($p < 0.05$) up to 139% and 155% at 6 and 8 hrs respectively of 40% and 80% of 24-h LD_{50} of *Ropalidia marginata venom* injection with respect to control. Further, it was improved up to 119% and 144% at 10 hrs with respect to control (Table 5 and Table 6 and Figure 17).

Contrary to this, ALP level was found to be decreased significantly ($p < 0.05$) up to 77% and 74% at 6 hrs of treatment with 40% and 80% of 24-h LD_{50} of purified *Ropalidia marginata* venom respectively. Further, it was found 74% and 80% at 10 hrs after the treatment with the same doses of the venom with respect to control (Table 5 and Table 6 and Figure 18).

Table 6. *In vivo* effect of 80% of 24-h LD_{50} purified *Roplidia marginata* venom toxins on different enzymes *i.e.*, acid phosphatase, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, lactic dehydrogenase, acetylcholinesterase in gastrocnemius muscle of albino mice.

Enzymes	Time in hrs.					
	0	2	4	6	8	10
ACP	0.86 ± 0.0816 (100)	1.23 ± 0.0816 (143)	1.31 ± 0.0816 (152)	1.4 ± 0.0816 (162)	1.34 ± 0.0816 (155)	1.24 ± 0.0816 (144)
ALP	0.61 ± 0.0816 (100)	0.58 ± 0.0816 (95)	0.54 ± 0.0816 (88)	0.49 ± 0.0816 (80)	0.48 ± 0.0816 (78)	0.53 ± 0.0816 (86)
GPT	2.53 ± 0.0816 (100)	2.71 ± 0.0816 (107)	2.8 ± 0.0816 (110)	2.81 ± 0.0214 (111)	2.81 ± 0.024 (111)	2.71 ± 0.816 (107)
GOT	0.42 ± 0.0124 (100)	0.50 ± 0.0124 (119)	0.54 ± 0.0816 (128)	0.56 ± 0.0816 (133)	0.48 ± 0.0124 (114	0.45 ± 0.0816 (107)
LDH	91.21 ± 0.0816 (100)	92.50 ± 0.179 (101)	93.21 ± 0.0816 (102)	94.11 ± 0.0816 (103)	93.46 ± 0.0816 (102)	92.95 ± 0.0816 (101)
ACHE	0.031 ± 0.0816 (100)	0.025 ± 0.0816 (80.6)	0.023 ± 0.0816 (74.5)	0.021 ± 0.0816 (67.7)	0.023 ± 0.0816 (74.19)	0.024 ± 0.0816 (77.4)

Values are mean ± SE of three replicates. Values in parentheses indicate per cent enzyme activity with respect to control taken as 100%. Significant ($p < 0.05$, Student t-test). Acid phosphatase (ACP) and Alkaline phosphatase (ALP): μ moles of p-nitrophenol formed/30minute/mg protein. Glutamate pyruvate transaminases (GPT): Units of Glutamate pyruvate transaminase activity/hour/mg protein. Glutamate oxaloacetate transaminase (GOT): Units of Glutamate oxaloacetate transaminase activity/hour/mg protein. Lactic dehydrogense (LDH): μ moles of pyruvate reduced/45minute/mg protein. Acetylcholinesterase (AchE): μ moles "SH" hydrolysed/minute/mg protein.

GPT level was found to be increased significantly; ($p = 0.05$) up to 110% at 10 hrs of 40% of 24-h LD_{50} and 111% at 8 hrs of 80% of 24-h LD_{50} of purified *Ropalidia marginata*venom injection concerning control. Further, it was improved up to 107% at 10 hrs concerning control (Table 5 and Table 6 and Figure 19).

Whereas GOT level was found to be increased significantly ($p < 0.05$) up to 135% at 6 hrs of 40% of 24-h L LD_{50} and 1114% at 8 hrs of 80% of 24-h LD_{50} of purified *Ropalidia marginata* venom injection with respect to control. Further, it was improved up to 107% and 110% at 10 hrs (Table 5 and Table 6 and Figure 20).

More specifically, LDH level was found to be increased significantly ($p = 0.05$) up to 102.9% and 103% at 6 hrs of treatment with 40% and 80% of 24-h LD_{50} of purified *Roplidia marginata* venom respectively. Further, it was found 101% and 101.6% at 10 hrs after the treatment (Table 5 and Table 6 and Figure 21).

More specifically, AchE level was found to be decreased significantly ($p < 0.05$) upto 87.3% and 67.7% at 6 hrs of treatment with 40% and 80% of 24-h LD50 of purified *Ropalidia marginata* venom in comparison to control respectively. Later on, it improved up to 93.61% and 77.4% at 10 hrs with respect to control (Table 5 and Table 6 and Figure 22).

4. Discussion

In the present, investigation effects of sub-lethal dose of paper wasp *Ropalidia marginata* venom toxins were observed in albino mice after administration of sub-lethal 40% and 80% 24 hrs LD_{50}. A significant elevation was observed in serum acid phosphatase, 141.9% at 6 hrs in comparison to control (Table 1 and Figure 5). Acid phosphatase is found in lysosomes and plays an important role in catabolism, pathological necrosis, autolysis, and phagocytosis [22]. Wasp venom may cause liver ischemia and hypoxia in toxin-treated experimental animals [23]. This increase in the level of acid phosphatase level might be due to the intoxication of liver cells and lysosomal disintegration. Disintegration of cells is responsible for leak out of various enzymes from liver and muscle cells into the blood circulation [24]. Same effect is also observed in malathion poisoning that significantly raised acid phosphatase and GPT levels respectively after treatment. But the alkaline phosphatase levels get significantly decreased after same treatment. Similarly, patulin a mycotoxin produced by a variety of molds *Aspergillus*, *Penicillium*, and *Byssochlamys* elevates level of acid phosphatase in the livers and kidneys and causes cell damage due to toxicity [25].

Alkaline phosphatase was found to be increased up to 119.9% at the 6^{th} hr of the toxin injection in comparison to control (Table 1 and Figure 6). This elevation may be due to cytolysis. In present study, ACP and ALP levels were also found to be increased significantly ($p < 0.05$) up to 141.9% and 119.9%, after n *vivo* venom toxin treatment (Table 1 and Table 2 and Figure 5 and Figure 6). This elevation in ACP and ALP is due to hemolytic activity of the venom toxin. Contrary to this the level of ALP was found to be reduced up to 74.19% and 80% in liver and gastrocnemius muscle at 6^{th} hr respectively in comparison to control

(Table 4 and Table 5; Figure 11 and Figure 18). This inhibition may retard the protein synthesis in tissues and release excess free amino acids in to circulation, thereby, increasing amino acid level in the serum.

The ALP enzyme is found in serum and organ tissues, especially liver, although significant concentrations are also found in kidney, skeletal muscle, and myocardium. ALP 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 [26]. It also causes break down of proteins and found in different forms, depending on where it originates. It also found in liver, gall bladder and bones. Main causes of its increase are malnutrition, metastasis of kidney, intestine and pancreas or a serious microbial infection. Lower levels of ALP are present in pancreas, spleen, and lung. Due to toxin induced damage of liver tissue activity of alkaline phosphatase in 40% of 24-h LD_{50} was treated mice found to be decreased by up to 78% at 6 hours of the in comparison to control (Table 1 and Table 3 and Figure 12). This reduction may be due to cytolysis. Alkaline phosphatase activity was found to be decreased up to 86% and 88% in liver and gastrocnemius muscle at 10 hrs respectively in comparison to control in 80% of 24-h LD_{50} (Table 3 and Table 5; Figure 11 and Figure 18). This decrease in the activity of alkaline phosphatase may retard the protein synthesis in tissues and release excess free amino acids in to circulation, thereby increasing the amino acid level in the serum.

Both acid phosphatase and alkaline phosphatase enzymes are mainly found in the blood, liver, plasma and intestine of human beings [27] [28]. Both these enzymes are detoxifying enzymes and their level increased after pesticide poisoning [29], toxins [30] and after a dose of 1,2,4-triasole derivative (3-(2-pyridil)-4phenyl-1,2,4-triasole-5-carboxilic acid) [31]. A similar increase in the level of alkaline phosphatase, acid phosphatase, alanine transaminase, creatine phosphokinase, and lactic dehydrogenase is reported after *Tityus serrulatus* venom administration by [32]. This alteration in serum enzyme is the result of the necrotic effects of the venom on different tissue especially the heart, liver, and blood cells [33]. It also caused a significant increase in serum levels of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, creatine phosphokinase, and lactic dehydrogenase, especially in the liver and heart. This significant increase in serum enzyme levels is also seen in case of liver diseases and its complications [34]. μ-agatoxins isolated from the venom of the Funnel-web spider (Agelenidae family), bind to glutamate glutamate receptors and do stimulation of neuro mediators release [35]. Huwenatoxin isolated from the venom of Brazilian wandering spider *Phoneutria nigriventer* are pore blockers of Na" channel [36]. These toxins interact with so-called receptor site 1 where classic inhibitor tetrodotoxin and saxitoxin as well as conotoxin bind [37]. Contrary to this, scorpion D-toxins retard inactivation of Na* channels and bind to site [38].

A significant elevation was observed in serum, liver, and gastrocnemius muscles lactic dehydrogenase activity, while a significant ($p < 0.05$) reduction was observed in acetylcholinesterase activity in albino mice. 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. Similarly, hornet's (*Vespa orientalis*) venom induces enzymatic changes in the liver after prolonged and repeated exposures. Wasp toxins cause hepatic damage in rats, *in vivo* that hinders detoxification process in hepatocytes. There is a possibility that paper wasp venom toxins cause cellular damage due to which most of the enzyme leaks out from liver and muscle cells into the circulation [34]. This might also altered cell permeability functions of myocardial tissue and liver and develops metabolic disorders and inflammation [39] [40].

Lactic dehydrogenase is cellular metabolic enzymes and occurs in small concentrations in plasma. An elevated level of LDH reflects high anaerobic power; the cellular processes depend more heavily on the mitochondrial capacity to regenerate ATP. Alterations mainly increase in LDH level showed regular physiological shedding of cells or tissue destruction due to toxicity [41]. 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 [42] High LDH level also shows stress and hypertension, which increases the rate of oxidation in animals. 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. LDH regulates the byproducts of mitochondrial oxidative or reductive stress conditions via the maintenance of lactic acid at normal level by converting it to pyruvate. Because LDH catabolize pyruvic acid the main end product of glycolysis and supply 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. In such cases pyruvic acid is reduced to lactic acid under the influence of lactic dehydrogenase. The lactate dehydrogenase (LDH) pathway has great potential for treating acidosis due to its ability to convert protons and pyruvate into lactate and thereby raise blood pH, but has been challenging to develop into a therapy because there are no pharmaceutical-based approaches for engineering metabolic pathways *in vivo* [43]. However, combined effects of heavy metal lead and hyperoxia involve activation of LDH to compensate for cellular changes in the cytoplasm [44].

However, in venom injected mice LDH activity in liver and gastrocnemius muscle was found to be increased up to 144% and 103% at 6 hrs respectively in comparison to control (**Table 4** and **Table 6** and **Figure 15** and **Figure 21**). High LDH level shows stress and hypertension and increase the rate of oxidation in animals. It is well known that pyruvic acid is the main end product of glycolysis in those tissues. When oxygen is supplied in abundance, but in those tissues where oxygen supply is insufficient or in anaerobic state, it causes more toxicity. For example, skeletal muscles lactic acid forms the usual end product glycolysis and pyruvic acid is reduced to lactic acid under the influence of lactic dehydrogenase [45]. Venom caused massive cellular toxicity in liver cells and caused significant alterations in cell permeability of myocardial, liver and smooth muscle

cells. These factors might be responsible for release of certain metabolic enzymes out of the cells in to the circulation Both LDH and ALP levels are used for diagnosis of liver disease, and monitor of various disorders. Venom toxins also affected nerve transmission; thereby activity of acetylcholine esterase was reduced up to 73.6 of 80% serum blood at 6 hrs of the venom injection in comparison to control (**Table 4** and **Table 6** and **Figure 22**). Its level in liver and gastrocnemius was also found to be decreased significantly up to 78% at 6 hr and 67.7% at 6 hrs respectively in comparison to control (**Table 4** and **Table 6** and **Figure 22**). It clearly indicates inhibition oh Ache activity by spider venom component. This inhibition of AchE activity causes the accumulation of acetylcholine molecule at the synaptic junction that result in the accumulation of acetylcholine and lead to prolonged activity of acetylcholine receptors. Therefore, due to the permanent stimulation of nerves and muscle cells, experimental mice displayed muscular paralysis and finally died [46]. However, in venom-injected mice, LDH level in the liver and gastrocnemius muscles was found to be increased up to 144% and 103% at 6 hrs respectively in comparison to control (**Table 4** and **Table 6** and **Figure 15** and **Figure 21**). *In vitro* treatment of purified venom inhibited the activity of acetylcholinesterase whereas the activity of phosphatase, and Na, K-ATPase enzyme remain unaltered. The *in-vitro* studies with this enzyme indicates that the venom bind the acetylcholinesterase enzyme competitively, thereby inhibiting its activity. An increase in acetylcholine at synaptic junction also decrease cholinergic transmission and increase the secretion of catecholamine which brings about more glycogenolysis [47]. Thus, inhibition in alkaline phosphatase and Na, K-ATPase in gastrocnemius *in vivo* treatment may be due to other reactions. The kinetic study clearly indicates the inhibition of acetyl cholinesterase by purified venom is competitive. More specifically, the above change was observed within 6 to 8 hrs of the venom injection. In present study, ACP and ALP level were also found to be increased up to 145.1% and 114.47%, *in vitro* venom treatment (**Table 1** and **Figure 5** and **Figure 6**). This elevation in ACP and ALP is due to haemolytic activity of the venom. But on the other hand, activity of AchE was found to be reduced up to 78% (**Table 1** and **Figure 9**).

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. Glutamate pyruvate transaminase, glutamate oxaloacetate and lactic dehydrogenase, all are cellular metabolic enzymes having no evident function in vertebrate plasma. Alteration in levels of these enzymes displays aberrant mitochondrial metabolism, which predisposes cells to oxidative stress and apoptosis. In skeletal muscle pyruvate is transaminase to alanine and transported to the liver. Inside liver glutamate pyruvate transaminase transfers the ammonia to the α-ketoglutarate and regenerates pyruvate. This is then utilized in the process of gluconeogenesis for glucose production [48]. Therefore, it functions as a link between carbohydrate and protein metabolism by catalyzing the conversion of alanine to pyruvate. The elevation in GPT level may be due to the stress that was created after *Rhipicephalus microplus* toxin injection. Therefore, saliva tox-

in-induced stress may be the causative factor for the elevation in GPT concentration [24]. It is evident that during stress conditions the energy requirement becomes high, which results in very high utilization of glucose and a massive breakdown of stored glycogen that leads to a decrease in glycogen level [48].

Alanine aminotransferase is elevated in serum under conditions of significant cellular necrosis and is used as a measure of liver function. High serum alanine amino transferase expression is associated with metabolic syndrome. After 8 hrs of venom toxin administration level of serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and lactic dehydrogenase was also found to be increased significantly up to 138.15%, 108.75%, 102%, in serum, liver and muscle respectively in comparison to control (**Table 1** and **Figure 8** and **Figure 9**). More often, glutamate-oxaloacetate transaminase activity significantly reduced the lipotoxic effects of palmitate, whereas knockdown of glutamate dehydrogenase. This aberrant transaminase metabolism fuels CAC dysregulation and oxidative stress [49]. Ischemic stroke results in excessive release of glutamate, which contributes to neuronal cell death. Here, we test the hypothesis that otherwise neurotoxic glutamate can be productively metabolized by glutamate oxaloacetate transaminase (GOT) to maintain cellular energetics and protect the brain from ischemic stroke injury. GOT enables the metabolism of otherwise neurotoxic extracellular Glu through a truncated tricarboxylic acid cycle under hypoglycemic conditions [50].

Acetylcholinesterase (AChE) is a cholinergic enzyme primarily found at postsynaptic neuromuscular junctions, especially in muscles and nerves. It immediately breaks down or hydrolyzes acetylcholine (AchE), a naturally occurring neurotransmitter, into acetic acid and choline. The acetylcholinesterase activity in the liver and gastrocnemius muscles was also found to be decreased significantly up to 89.02% and 77.4% at 10 hours in comparison to control (**Table 4** and **Table 6** and **Figure 16** and **Figure 22**). This inhibition of acetylcholinesterase activity results in much longer stay of acetylcholine molecules at the synaptic junctions. It 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. Toxic substances mainly pesticides affect acetylcholinesterase activity in mice [51]. Increased acetylcholine esterase activity produced by the administration of an aqueous extract of the seed kernel of *Thevetia peruviana* and its role on acute and subchronic intoxication in mice [52]. Acetyl cholinesterase activity in the serum, liver and gastrocnemius muscles was altered due to secondary effects of the albino mice *In vivo* condition. In present study serum acetyl cholinesterase was found to be reduced up to 84.41% at 10 hours of the venom injection in comparison to control (**Table 4** and **Table 6** and **Figure 16** and **Figure 22**). Similar effects were noted in acetyl cholinesterase levels after 8 hrs of administration of sub-lethal dose of *Polistes flavus* venom toxins in albino mice in blood serum [53]. Organophosphate (OP) pesticides also inhibit acetylcholinesterase (AChE) activity. Similarly, diazinon poisoning also influence acetylcholinesterase and butyrylcholinesterase level in affected animals and

man [54]. Similarly, venom toxins from *Nemopile manomurai* Jellyfish inhibited acetyl cholinesterase (AChEs).

Serum acetyl cholinesterase was found to be reduced up to 84.41% at 10 hours of the venom injection in comparison to control (**Table 4** and **Table 6** and **Figure 16** and **Figure 22**). Significant decreases in RBC and plasma AChE were observed in the exposed group in comparison with controls [55].

Hymenoptera stings may cause both local and systemic allergic reactions due to the synthesis of IgE and even life-threatening anaphylaxis. The most common reactions (up to 26%) are edema, erythema, pain, and witness. However, a systemic reaction develops in 0.3% - 7.5% of the adult population of beekeepers. Along with pharmaceutical drugs and foods, Hymenoptera venom is one of the most common causes of anaphylaxis in humans. Venom immunotherapy (VIT) is the most effective method of treatment for people who had SSR, which is shown to be effective even after discontinuation of the therapy [56]. The development of peripheral tolerance is the main mechanism during immunotherapy but it is a complex process. It is mediated by the production of blocking IgG/IgG4 antibodies that may inhibit IgE-dependent reactions through both high affinity (FcεRI) and low affinity (FcεRII) IgE receptors on mast cells, basophils and B cells [55]. The main goal of VIT is to induce a change from the proinflammatory Th2 response to the Th1 response [16]. Antibody administration is the most effective method of treatment [57]. Wasp venom toxins also caused liver ischemia and hypoxia and impose oxygen deficient state. This may led to dissolution of glycogen inside liver that increased the glucose level and its catabolism very rapidly. Finally, it may lead to increase in production of pyruvate which renders significant increase in LDH level and death of hepatocytes. Finally toxin interactions with enzymes and its receptors imposed biological effects and few of them were noted in behaviour of experimental animals. As prolonged activation of acetylcholine receptors caused permanent stimulation of nerves and muscle cells that was resulted in muscular paralysis in animals.

5. Conclusion

Wasps, members of the order hymenoptera, use their venom for predation and defense. These massively sting passerby and travellers to make territorial defence. Wasp's venom is a cocktail of many allergens, enzymes, bioactive peptides, amino acids, biogenic amines, and volatile substances. Due to presence of highly active components wasp stings cause both local and systemic allergic reactions and even life-threatening anaphylaxis mediated by immunoglobulin E. Usually, the systemic reactions include skin, gastrointestinal, respiratory, and cardiovascular symptoms, which may develop separately or in combination. Anaphylaxis to multiple wasp sting cause terrifyingly rapid death, with initial cardio respiratory arrest within 30 - 45 min of the venom sting and unavailability of clinical and therapeutic aid. However, for neutralization of pathological effects administration of antiserum is highly useful. Present study will be helpful in understanding of the patho-physiology of wasp venoms toxins and its interac-

tion to key metabolic enzymes whose restoration can help to normalization of envenomated patients. This enzyme-toxin interaction could be used for drug design and innovative therapeutic discoveries.

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Authors' Contributions

Ravi Kant Upadhyay and Simran Sharma were responsible for conception, experiments, writing and revising the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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