Journal of Biosciences and Medicines, 2023, 11, 55-75
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Effect of Purified Paper Wasp *Ropalidia marginata* Venom Toxins on Different Biomolecules in Mice Serum

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How to cite this paper: Sharma, S. and Upadhyay, R.K. (2023) Effect of Purified Paper Wasp *Ropalidia marginata* Venom Toxins on Different Biomolecules in Mice Serum. *Journal of Biosciences and Medicines*, **11**, 55-75.
https://doi.org/10.4236/jbm.2023.115005

Received: March 1, 2023
Accepted: May 8, 2023
Published: May 11, 2023

Abstract

This study evaluated the effects of purified paper wasp *Ropalidia marginata* venoms on various biomolecules in the blood serum of albino mice. Changes in the concentration of some important macromolecules, *i.e.*, proteins, free amino acids, uric acid, cholesterol, pyruvic acid, total lipids and glucose were noted down. These alterations were measured after intraperitoneal injection of 40% and 80% 24-hour LD_{50} purified *Ropalidia marginata* venom toxin. Serum total protein levels were found to decrease to 78% after 6 hrs, while serum free amino acid levels were significantly increased to 117% 6 hrs after venom injection compared to control. It was also found that serum uric acid levels increased to 138% after 8 hrs of venom injection compared to control. The increase in serum cholesterol *i.e.* (101% and 106%) and pyruvic acid increased significantly to a maximum value of 106% after 6 hrs of treatment at 40% LD_{50}. Glycogen levels in the gastrocnemius muscle were found to decrease significantly (p-0.05) to 43% and 92% at LD_{50} after injection of purified *Ropalidia marginata* venom after 8 h and 80% at LD_{50} compared to control. Moreover, up to 71% and 81% were obtained at 10 hrs of treatment with the same dose. In the present study, the purified toxins significantly changed the levels of biomolecules in blood serum, indicating their wider effects on cellular physiology due to toxic effects and stress on the animal. These toxins can be good antigens and stimulate immune responses in experimental mice.

Keywords

Paper Wasp, Venom and Toxin, Lethal Effects, Blood Serum, Biomolecules

1. Introduction

Wasps are hymenopteran insects belonging to the genus Vespa. Hymenoptera

insects are giant natural predators that play an important role in pollination, natural pest control, and biodiversity [1]. Like wasps, hornets are found almost everywhere in the world. The Asian giant wasp *Vespa mandarinia*) is the world's largest social wasp, building communal nests by chewing wood into paper pulp. To date, more than 30,000 species of damsel wasps have been reported worldwide. Of these, 22 recognized species belong to the genus Vespa [2]. Wasps defend their nests very aggressively and are quick to use stings more dangerous than bees after the slightest disturbance [3]. Venom sting possess peptides which cause strong haemolytic activity. Wasp venom toxin peptides have also shown catalytic activity and formed pores in biological membranes [4]. Stings contain large amounts (5%) of acetylcholine and cause severe muscle pain. Wasp venom causes allergen-specific reactions and triggers an immune response to more important physiological changes [5]. The Asian giant oyster (*Vespa mandarinia*) poisons very quickly and causes death in humans [6]. Its toxins cause multiple organ failure, leading to death. Dialysis allows toxins to be quickly removed from the bloodstream. In a severe attack, allergic individuals face anaphylactic shock and die if not treated early [7] [8]. Wasp venoms contain important enzymes such as hyaluronidase, phospholipase A2, metalloendopeptidase, etc. However, some neurotoxic peptides (eg, pompilidotoxin and dendrotoxin-like peptide) and proteins (e.g., insulin-like peptide-binding protein) appear to be specific to individual wasp venoms. In contrast, several proteins, such as poison allergen 5 protein; venom acid phosphatase and various phospholipases appear to be relatively more specific for social wasp venom [9]. The insecticide is a rich source of peptides that could be used in drug development and innovative drug discovery [10].

Hymenoptera venom is a complex mixture of many substances such as toxins, enzymes, growth factor activators and inhibitors. It contains a few biologically important enzymes *i.e.*, phospholipases, acid phosphatase, proteases and therapeutically important peptides such as Mastoparan-C (MP-C), capin A, apamin, mast cell degranulation peptide (MCD), bradykinin, AMPs with various therapeutic potential. *Neoponeravillosa* venom induces haemolysis in human erythrocytes and also stimulates the release of both pro-inflammatory cytokines and anti-inflammatory cytokine release by murine macrophages [11]. Wasp venom contains the enzyme phospholipase, acid phosphatase and various phospholipases which appear to be relatively more specific for social wasp venom. Wasp venom binds to a large number of proteins and receptors and causes pathophysiological changes in victims. These enzymes cause the breakdown of cell membranes and hypersensitivity reactions, including life-threatening anaphylaxis. In addition, phospholipase A2 is an important component of bee venoms, while phospholipase A1 (PLA1) is very abundant in wasps and ants. Common components of both solitary and social wasp venoms are hyaluronidase, phospholipase A2, metalloendopeptidase. These enzymes trigger an immune response, causing an IgE reaction in susceptible individuals [12]. Some neurotoxic pep-

tides (e.g., pompilidotoxin and dendrotoxin-like peptide), proteins (e.g., insulin-like peptide-binding protein) and allergens also found in individual wasp venoms. Like the venom found in most venomous animals, parasitoids contain complex proteins with potential agrochemical and pharmaceutical applications. Hymenoptera are a reservoir of poisonous protein toxins that are involved in various biological processes such as pain, paralysis, allergic reactions and antimicrobial activity [13].

Hymenoptera insects inject large amounts of venom into the victim, causing massive inflammation, swelling and pain. It prevents breathing due to extensive swelling of the tracheal region [14] and high mortality and systemic reactions after poisoning [15]. Usually, a large number of victims have survived a bee sting, but sometimes a sting in the neck also prevents breathing due to swelling of the trachea. The severity of the venom increases as the amount of venom injected increases [16]. Mortality increases with age [17] [18] the poison has little effect on liver and kidney cells that make metabolic changes in the body [19], B lymphocytes, secrete group immunoglobulins and release interferons. Each immunoglobulin released by a B lymphocyte recognizes different epitopes and binds selectively [20] [21] [22] [23] [24]. Melittin is an important toxic component found in bee venom and has versatile biological activity [25]. The present research analysed the effects of *Ropalidia marginata* venom toxins on various biomolecules in albino mice. This study shows that toxin induced inhibition of certain biomolecules and enzymes lead to the failure of major metabolic pathways related to insect growth. If toxic effects persist for longer period insect will die and no progeny will proceed further. Hence, toxin could be used for safer control of insects and have many biomedical applications.

2. Methodology

2.1. Collection of *Ropalidia marginata* Toxins

The living specimens of paper wasp *Ropalidia marginata* was collected from rural areas of 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 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 supernatant was separated out. Total protein contents were estimated in the different supernatants according to the Lowry's (1951) [26].

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 between 5 ml/minute was maintained by a continuous buffer supply in a cold room. Elution of the venom proteins through gel filtration column was done in a cold room at the flow rate of 5 ml/minutes. Total 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 graph; absorbance in each fraction was determined at 280 nm using Shimadzu spectrophotometer (UV 2001 PC). Further, absorbance of same fractions was taken at 640 nm after protein estimation by Lowery (1951) [26].

2.3.1. 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 *Ropalidia marginata* venom toxins. The protein content eluted in each fraction was determined by using the method of Lowry *et al.*, (1951) [26].

2.3.2. Molecular Weight Determination of Purified Venom Proteins

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 Sepharose CL-6B gel column as done previously at the same flow rate. A calibration curve was drawn between Ve/Vo log M and with the help of calibration curve range of molecular weight of different protein in the purified *Ropalidia marginata* protein/toxins was determined.

2.3.3. Lyophilization of Eluted Venom Protein

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

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

The albino mice were injected subcutaneously with the purified venom toxins of different serial concentration and LD_{50} was determined at the intervals of the 24 hours. Deformities such as paralysis and neurotoxic effects were also recorded. Six albino mice injected with serial concentration of the venom toxins to determined LD_{50}. Mortality was determined by using Abbot's formula. The LD_{50} val-

ues 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.

2.4.1. Determination of Blood Bio-Molecules

1) Isolation of blood serum:

Both control and tested albino mice were bled at the same time for obtaining blood serum. Freshly drawn blood were taken directly into a clean glass test tube without adding any coagulants. The blood was allowed to clot in cold and the clot was carefully detached from the wall of the tube by applying a clean applicator stick around the innersurface of the tube. It was centrifuged immediately in a cooling centrifuge at top speed 15,000 × g for removing any particulate matter from the pellet. Fresh serum was collected and stored at 4˚C for experimental purpose. It was used for the analysis of different biochemical parameters.

2) Determination of serum total protein:

Estimation of the total protein in the serum was carried out by Lowry's method [26] (1915). 0.20 ml of serum was taken and 0.3 ml of distilled water was added to it. Then 0.50 ml of freshly prepared alkaline copper solution (Reagent C) was added. Reagent C was prepared by adding of 50.0 ml of reagent A (2% sodium carbonate in 0.1 N NaOH) and 1.0 ml of reagent B (1% sodium potassium tartrate, 0.5% copper sulphate mixed in 1:1 ratio at the time of experiment). The reaction mixture was kept for 10 minutes at room temperature: Then 0.50 ml of Folin-Ciacalteu reagent (diluted 1:2 ratio with distilled water at the time of experiment) was added to it. Contents were mixed well. After 15 minutes a blue color was developed which was measured at 600 nm. Standard curve was prepared by using different known concentrations of bovine serum albumin (BSA) the total serum protein was expressed in mg/100ml of blood serum.

3) Determination of total free amino acid:

Changes in the level of free amino acids in blood serum of albino mice was determined according to the method of Spies (1957) [27]. For this purpose, 0.10 ml of serum was taken in a clean glass test tube, 0.1 ml of distilled water and 2.0 ml of ninhydrin reagent were added to it and mixture was mixed thoroughly. Ninhydrin reagent was prepared by mixing 1.0 gm ninhydrin in 25 ml of absolute ethanol and 0.04 gm of stannous chloride in 25 ml of citrate buffer (pH 5.0). The reaction mixture was kept in boiling water for 15 minutes. Now the contents were allowed to cool and after cooling 2.0 ml of 5.0% ethanol was added to it. A violet colour was developed which is measured at 575 nm on visible spectrophotometer (Systronics). Standard curve was drawn using the known amount of glycine. The value of free amino acid was expressed as mg/100 ml of serum.

4) Determination of serum glucose:

Changes in serum glucose level was measured according to the method of Mendel *et al.*, (1954) [28]. For this purpose 1.0 ml of serum was taken and to it 8.0 ml of distilled water was added. Now in each tube 0.50 ml of 0.66N sulphuric

acid and 10% sodium tungustate solution were added. Contents was allowed to react and left stand for 10 minutes to ensure complete protein precipitation. Contents was filtered and precipitate was discarded. Three test tubes were taken. In first tube 4.0 ml of the filtrate was poured, to the second 4 ml of working uric acid standard (20 grams of uric acid was dissolved into 250 ml of distilled water) while 4.0 ml of distilled water to the third. 1.0 ml of 14% sodium carbonate solution and 1.0 ml of uric acid reagent (10 grams of sodium tungstate and 2.0 grams of anhydrous disodium phosphate were dissolved in a flask containing 30 ml of distilled water. In a second flask gradually 2.5 ml concentrated sulphuric acid was added to 50 ml of distilled water and it was cooled. Then added dilute sulphuric acid solution to the sodium phosphate solution and refluxed this mixture for one hour. This was then cooled and diluted to 100 ml with distilled waterto each tube and was left stand for 15 minutes at room temperature. The optical density was read at 680 nm setting the instrument to zero density with the solution containing only water and the reagent.

5) Determination of serum pyruvic acid:

Changes in level of pyruvic acid was determined according to the method of Freidman and Haugen (1943) [29]. For estimation of serum cholesterol 0.05 ml of serum was added to 5.0 ml of alcoholic potassium hydroxide solution (added 6.0 ml of 30% KOH solution to 94 ml absolute alcohol). Contents was shacked well and incubated in a water bath at 37˚C for 55 minutes. It was allowed to cool at room temperature and then 10 ml of petroleum ether was added and mixed well. Now 5.0 ml of water was added and shacked vigorously for 1 minute. Contents was centrifuged at slow speed (1200) × g for 5 minutes to make the clear layers of petroleum ether and water. A 5.0 ml aliquot of the petroleum ether was transferred to a dry test tube and placed in a 60˚C water bath. Solvent was evaporated by using stream of air over the solution. Standard cholesterol solution was prepared by dissolving 100 mg of dry cholesterol in sufficient absolute alcohol to make volume up to 250 ml. This solution contained 0.4 mg cholesterol in 1.0 ml. A 5.0 ml sample of standard cholesterol solution was mixed with 0.30 ml of 33% KOH solution. Shacked well and incubated in water bath at 37˚C for 55 minutes. Contents was cooled at room temperature and to it 10 ml of petroleum ether was added and mixed well. Now 5.0 ml of distilled water was added and shacked vigorously for 1 minute. It was centrifuged at slow speed (122 × g) for 5 minute or until the emulsion broken into two clear layers. After centrifugation 1, 2, 3 and 4 ml aliquot of the petroleum ether layer was taken into four separate test tubes and evaporated to make dry. These standards contained the equivalent of 200, 400, 600 and 800 mg of cholesterol/100ml. Now test tubes was arranged for testing the cholesterol contents in the test tubes. An empty test tube (for bank) then four test tubes for standard containing dried sample followed by unknowns was set. Now 6.0 ml of modified Leibermann-Burchard reagent (2.0 ml of concentrated HSO_4 was added to 40.0 ml of chilled acetic anhydride, shacked well and kept cold for 9 minutes. After this 20 ml of glacial acetic acid was added

and warmed the mixture to room temperature) was added to each test tube. The test tubes were shacked and returned to water bath. After 30 minutes optical density was determined at 620 nm setting the instrument to read zero density with blank.

6) Determination of serum uric acid:

Changes in serum uric acid level was determined by the Cyanide free method of Folin (1933) [30]. For this purpose, blood serum was deproteinized with 5% CA containing 0.10% silver sulphate and centrifuged at 10,000 × g for 10 minutes. Then 1.0 ml of dinitrophenylhydrazine was added to 0.10 ml deproteinized serum to react at room temperature for 15 minutes. Same procedure was carried out with the dilute pyruvic acid standard solution. Now 3.0 ml of xylene was added, air was passed and left the mixture for 2 minutes. After setting reaction mixture, the lower layer was discarded by means of a pipette. Then 6.0 ml of 10% sodium carbonate was added and mixed again by bubbling the air through the mixture for 2 minutes. After permitting the mixture to settle, 5.0 ml of the aqueous layer was taken in other test tube and added 5.0 ml of 1.5N-NaOH solution. It was mixed thoroughly and left for 10 minutes. Absorbance was read at 520 nm after setting the instrument at zero absorbance with blank containing 5.0 ml of 10% sodium carbonate and 5.0 ml of 1.5N-NaOH. The serum pyruvic acid was measured in terms of mg/100ml blood serum.

7) Determination of serum cholesterol:

Changes in serum cholesterol level was measured according to the method of Abell *et al.*, (1952) [31]. For this purpose, 500 ul of clear serum was mixed with a mixture of choloform and methanol (2:1 v/v). The mixture was allowed to suspend and kept for 2 hr at room temperature. It was filtered with Whatmann paper no. 1. The residue was resuspended in the same volume of mixture for one hour and again filtered the supernatant. Both filtrates were mixed with equal volume of 0.6% NaCl (w/v). The separatory funnel containing above mixture were kept in dark for 12 hr at room temperature. The upper layer of solvent (Chloroform + Methanol) was collected and unsaponified part kept unused. The contents of lower layer were allowed to evaporate by keeping it in oven at 60˚C. Total lipid contents were weighted at the end and expressed in mg/100ml of blood serum.

2.4.2. Determination of Serum Glucose

Changes in serum glucose level were measured according to the Mendel *et al.*, (1954) [32]. For this purpose, blood serum was deproteinized with 5% TCA containing 0.1% silver sulphate. The mixture was centrifuged at 10,000 × g for 10 minutes. In this 0.50 ml deproteinized supernatant, 4.5 ml of H_2SO, was added and mixed thoroughly. Contents were boiled in water bath for 6 minutes and the mixture was allowed to cool at room temperature. The pink colour obtained was read at 520 nm. The blank was set by using 0.5 ml of 5% TCA containing 0.1% silver sulphate and 4.5 ml of H_2SO_4. The glucose was level expressed as mg/100ml of blood serum.

3. Results

3.1. Solubilization of *Ropalidia marginata* Venom Toxins

Before extracting poisonous toxins, it was homogenized, 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 insulation and cleaning; Ropalidia marginata was 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 × G and the supernatant was carefully separated. It was loaded onto a Sepharose CL-6B 200 column to separate the venom toxins. The elution pattern of venom toxin homogenate 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 1, Figure 2). In addition, the concentration of *Ropalidia marginata* toxin was determined in each test tube using the method of Lowrey (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 fraction numbers 61 and 67 (Figure 1, Figure 2). Both peaks were eluted with PBS buffer (pH 6.9). The total yield of poisonous toxins in the eluted fractions was 76.8%.

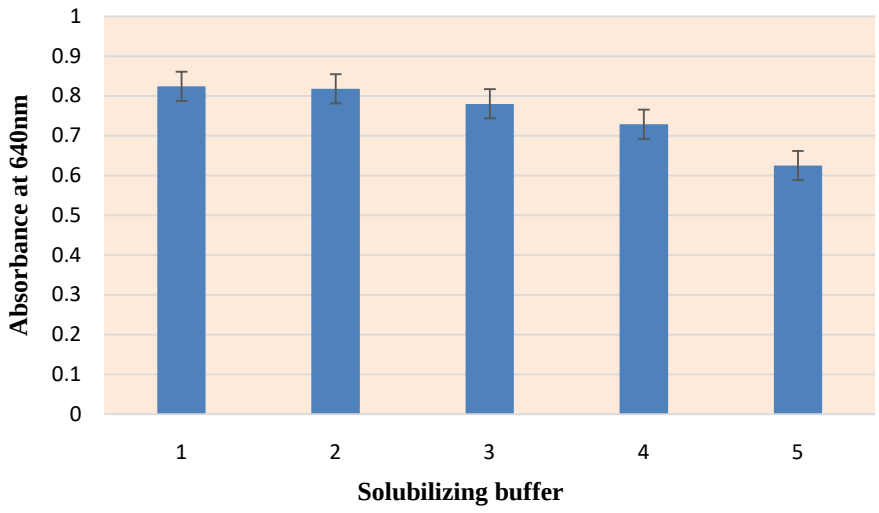

Figure 1. Solubilization of Ropalidia marginata in different buffers. Absorbance of solubilized protein was taken at 640 nm. Solubilizing buffers on X-axis are (1) Triton X 100 (0.01%), (2) Tris + EDTA (0.1 Mm) (3) PBS buffer (4) TCA 5% and (5) Absolute alcohol.

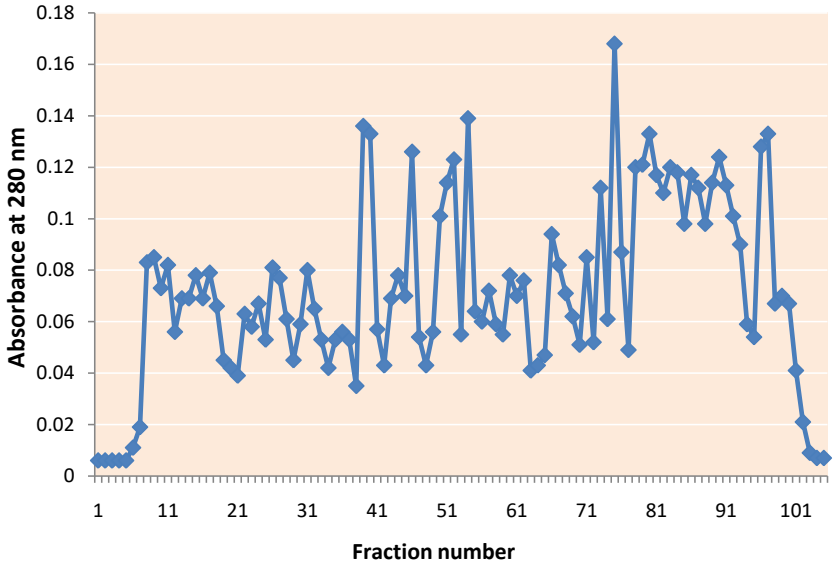

Figure 2. Elution pattern of PBS extractable proteins of *Ropalidiamarginata wasp* chromatographed on a Sepharose CL-6B 200 column Absorbance taken at 280 nm.

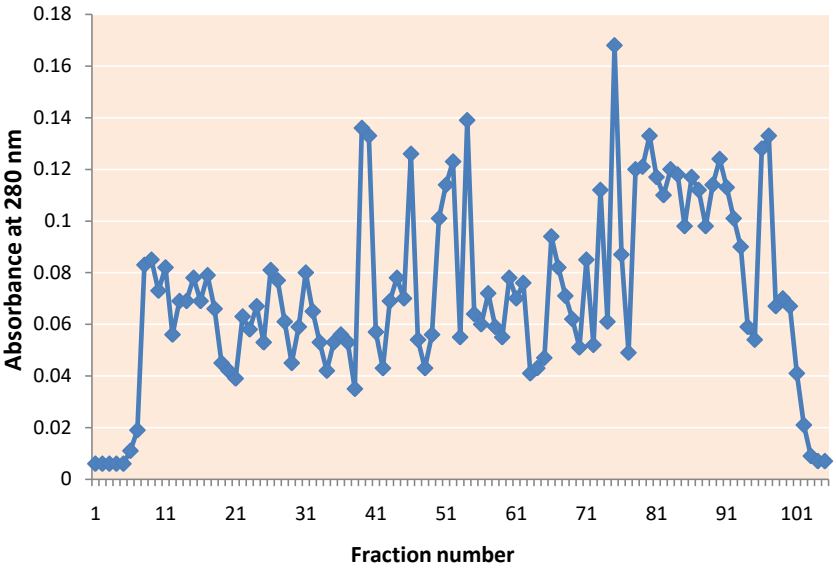

Figure 3. Elution pattern of PBS extractable proteins of *Ropalidia marginata* wasp chromatographed on a Sepharose CL-6B 200 column. Absorbance taken at 640 nm.

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 against albino mice (Mus musculus). Wasp venom proteins obtained by lyophilization of two pounds caused 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.

In this section, the effects of purified *Ropalidia marginata* venom on various biomolecules 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.

It was found that total protein levels in the serum of albino mice were significantly reduced by up to 88% and 73% at 40% and 80% of the LD_{50} compared to the control within 6 hrs. It later recovered slightly to 90% and 78% at 10 hrs compared to the control group (Figure 5).

It was found that free amino acid levels in the serum of albino mice were significantly elevated to 114% of the 24-hour LD_{50} of purified *Ropalidia marginata* after six hours. Poisons, while 80% of the 24-hour LD_{50} caused a significant increase of 124% after 10 hrs. In addition, it recovered by 100% and 125%, respectively, compared to the control after 10 h of treatment (Figure 6). The 24-hour LD_{50} of purified *Ropalidia marginata* venom toxins were found to increase serum uric acid by 122% and 138% by 40% and 80% compared to the control group (Figure 7).

Similarly, a marginal increase in serum cholesterol levels, *i.e.*, 101% and 106%, was observed at 40% and 80% of the 24-h LD_{50} of purified *Ropalidia marginata* venom toxins at 6 h compared to the control group.

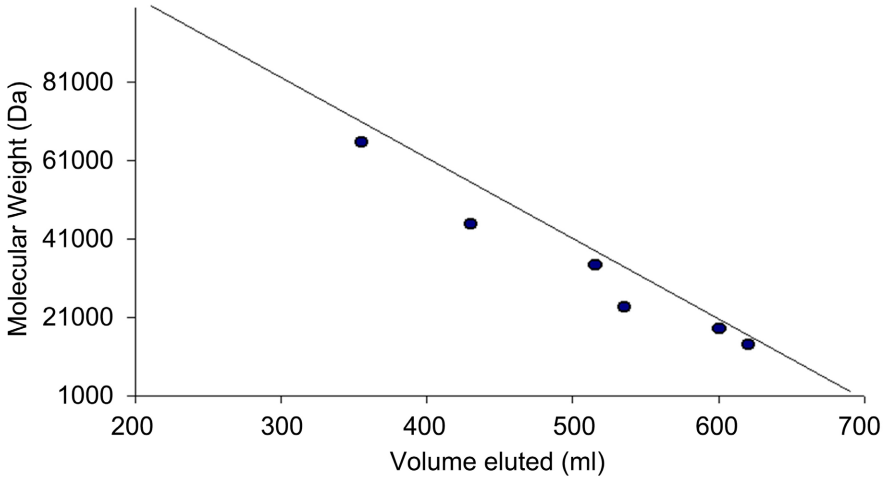

Figure 4. Standard proteins chromatographed on Sepharose CL-6B 200 column for determining the molecular weights peptides isolated from *Ropalidia marginata.* Proteins used were bovine albumin mol. wt. 66,000, egg albumin mol. wt. 45,000, pepsin mol. wt. 34,700, trypsinogen mol. wt. 24,000, beta lactoglobulin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

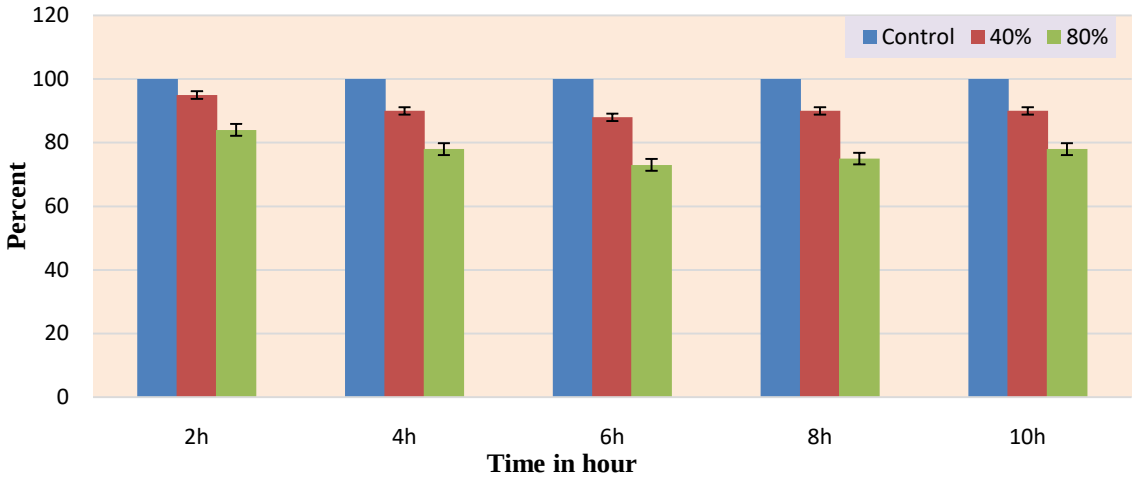

Figure 5. *In vivo* effect of 40% and 80% of 24 h LD_{50} of purified venom toxins of paper wasp *Ropalidia marginata* on serum total protein of albino mice.

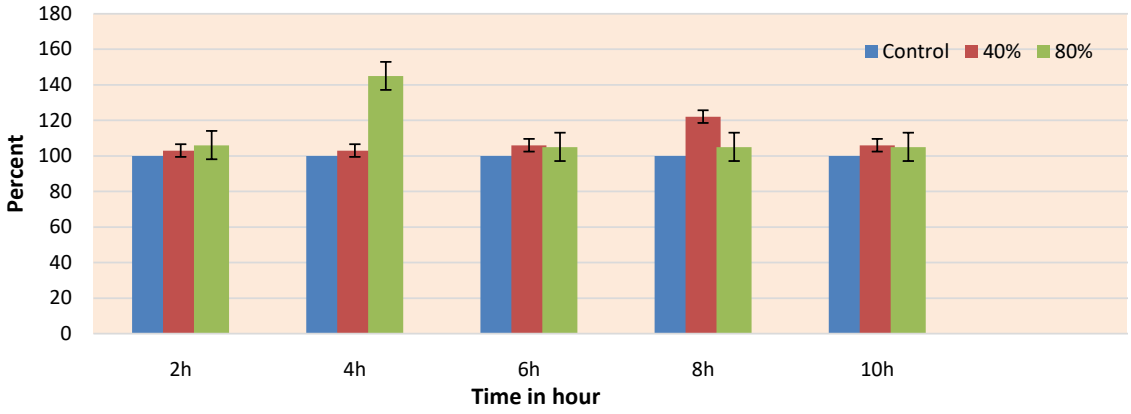

Figure 6. *In vivo* effect of 40% and 80% of 24 h LD_{50} of purified venom toxins of paper wasp *Ropalidia marginata* on serum uric acid of albino mice.

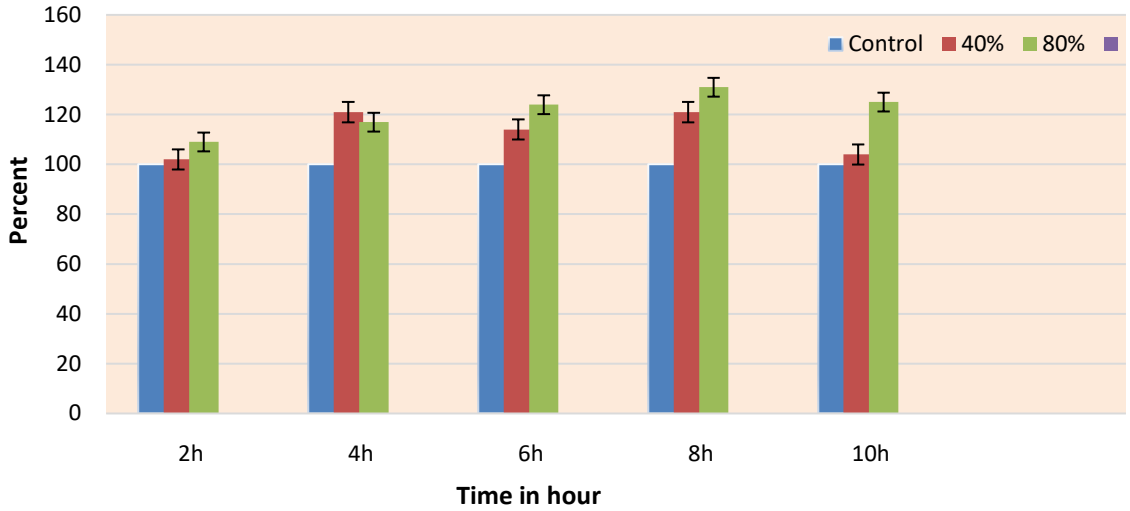

Figure 7. *In vivo* effect of 40% and 80% of 24 h LD_{50} of purified venom toxins of paper wasp *Ropalidia marginata* on free amino acid of albino mice.

In addition, it was 101% and 102% compared to the control after 10 hrs of treatment (Figure 8). Serum pyruvic acid increased significantly to a maximum value of 106% at 6 h after treatment with 40% LD_{50}, while it was 108% at 10 hr with 80% purified venom compared to the control group. Again, it normalized to 104% and 108% at the same doses compared to control (Figure 9).

A significant increase in serum total lipid levels was 40% and 80% of purified venom at 8 h treatment with a 24 h LD_{50} of 120% and 146%, respectively, compared to the control. Later it turned out that in the 10 hrs of treatment it was 107% and 134% (Figure 10). This resulted in a 107% increase in serum glucose after 10 hours, which is 40% of the 24-hour LD_{50} injection. It then increased to 118% 80% of the 24th LD_{50} at the 8 h dose, and this increase (118%) was sustained until the 10th hour (Figure 11).

The effects of purified *Ropalidia marginata* venom toxins were observed on

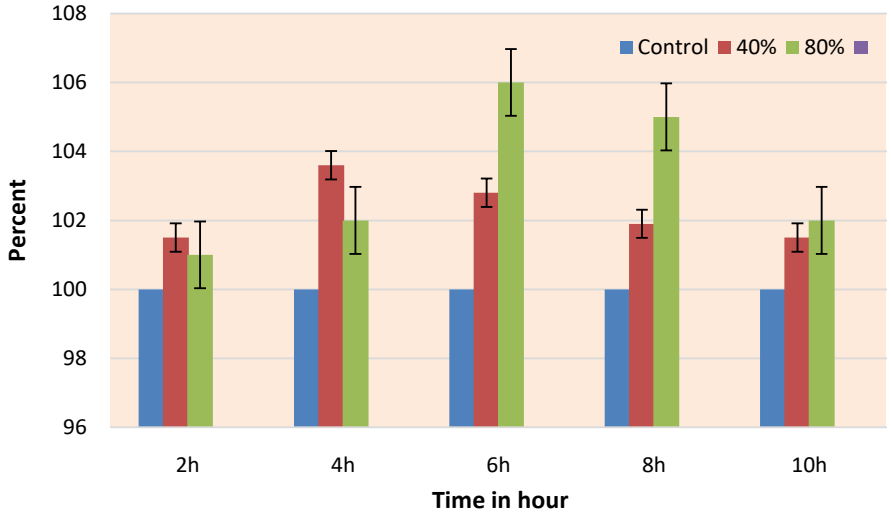

Figure 8. *In vivo* effect of 40% and 80% of 24 h LD_{50} of purified venom toxins of paper wasp *Ropalidia marginata* on cholesterol of albino mice.

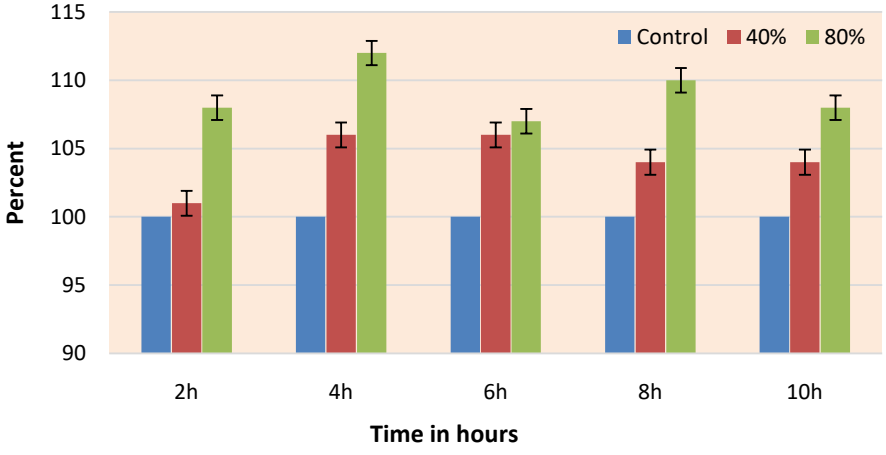

Figure 9. *In vivo* effect of 40% and 80% of 24 h LD_{50} of purified venom toxins of paper wasp *Ropalidia marginata* on serum pyruvic acid of albino mice.

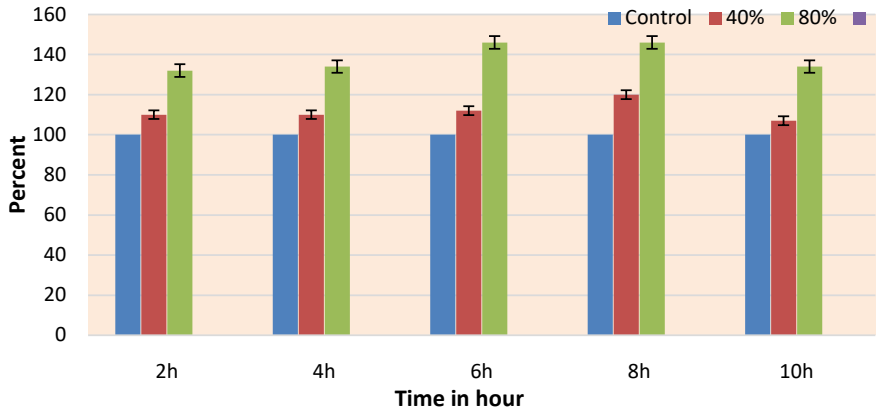

Figure 10. *In vivo* effect of 40% and 80% of 24 h LD_{50} of purified venom toxins of paper wasp *Ropalidia marginata* on serum total lipid of albino mice.

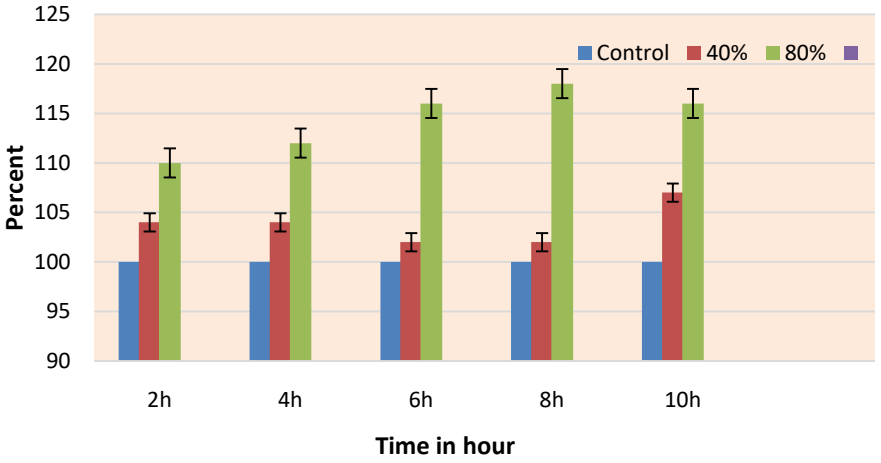

Figure 11. *In vivo* effect of 40% and 80% of 24 h LD_{50} of purified venom toxins of paper wasp *Ropalidia marginata* on serum glucose of albino mice.

the liver, rectus abdominis muscle, gastrocnemius muscle, atrium and ventricle of albino mice. Changes in glycogen levels were assessed after venom injection at 40% and 80% of the 24-h LD_{50} value at different time points, after which changes in glycogen levels were observed.

Liver glycogen levels were found to decrease significantly ($p \leq 0.05$) to 74% 4 h 40% 24 h LD_{50} and 86% and 8 h 80% 24 h LD_{50} in *Ropalidia marginata* venom injection compared to control. Moreover, it was obtained up to 67% and 92% in 10 hrs (Figure 12).

A significant decrease in rectus abdominis muscle glycogen was 73% at 6 hrs 40% 24 h LD_{50} and 88% at 8 h 80% 24 h LD_{50} n 88% compared to the control group. It was later recovered to 82% and 95% after 10 hrs (Figure 13).

Glycogen levels in the gastrocnemius muscle were found to be significantly ($p \leq 0.05$) decreased by 43% and 92% at 8 h LD_{50} and 80% h LD_{50} of injection of purified *Ropalidia marginata* venom compared to control. In addition, up to 71% and 81% were obtained after 10 hrs of treatment with the same dose (Figure 14). Significant ($p \leq 0.05$) decrease in atrial glycogen level was 58% and 71% at 8 h

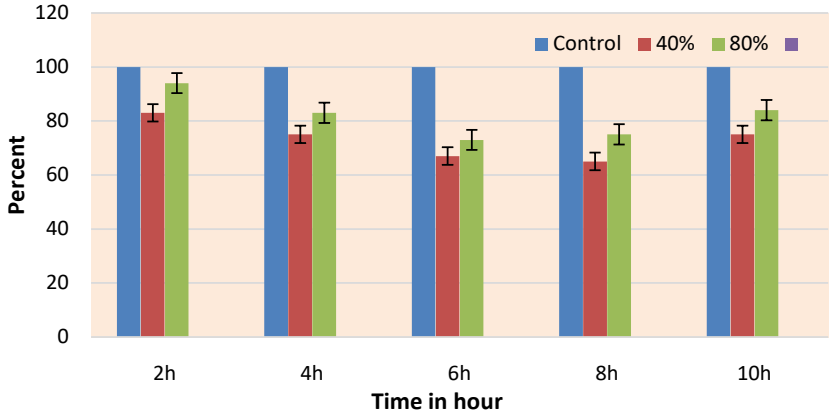

Figure 12. *In vivo* effect of 80% of LD_{50} of purified *Ropalidia marginata* venom toxins on glycogen level in liver of albino mice.

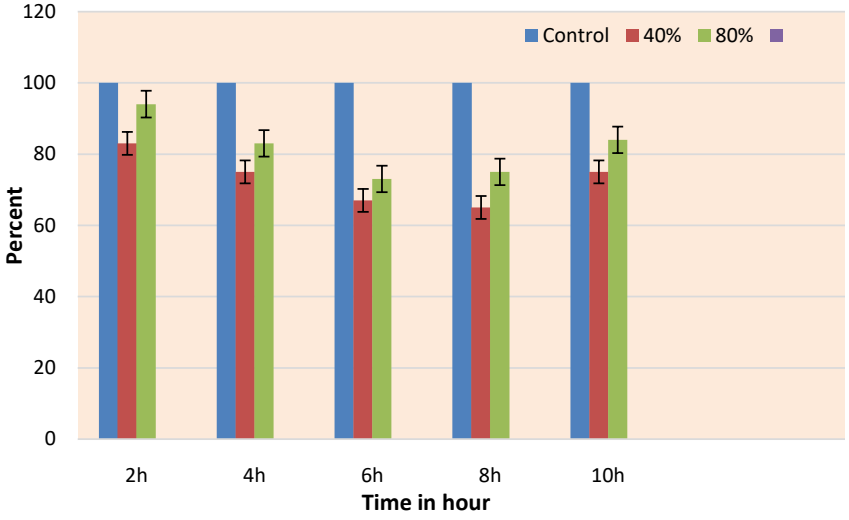

Figure 13. *In vivo* effect of 80% of LD_{50} of purified *Ropalidia marginata* venom toxins on glycogen level in Rectus abdominus of albino mice.

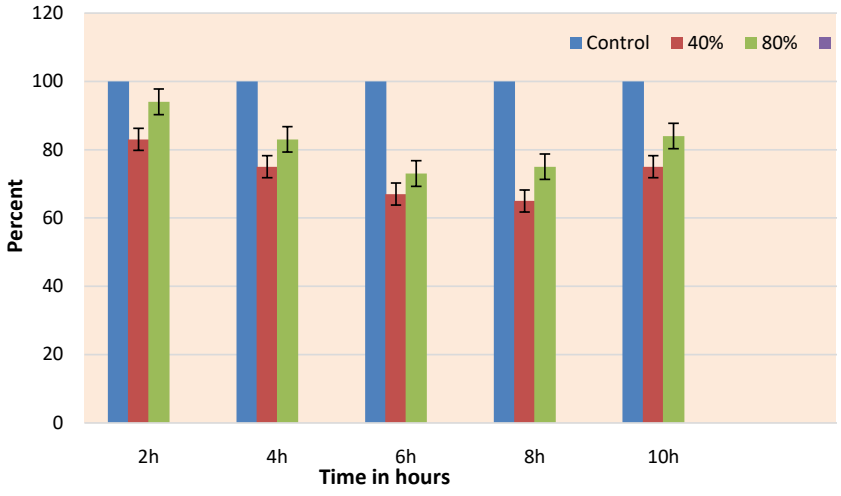

Figure 14. *In vivo* effect of 80% of LD_{50} of purified *Ropalidia marginata* venom toxins on glycogen level in gastrocnemius of albino mice.

40% and 80% at 24 h LD_{50} of purified *Ropalidia marginata* venom compared to control. Later, it was 69% and 81% at 10 hrs (Figure 15).

Similarly, ventricular glycogen levels were significantly ($p \leq 0.05$) reduced to 67% at 6 h 40% 24 h LD_{50} and to 84% at 80% LD_{50} 80% 6 hrs (Figure 16).

4. Discussion

In this study, purified paper wasp *Ropalidia marginata* venoms produced a significant LD_{50} of 40% and 80% in the bio-molecule after 24 hrs of injection. Albino mice given a sub-lethal dose of purified *Ropalidia marginata* venom were found to have a 78% reduction in total serum protein after 6 hrs. It indicates the breakdown of proteins for energy and can also lead to the formation of amino acids. This is the main reason why serum free amino acid levels were found to be significantly increased by up to 117% compared to control after 6 hrs of venom injection. The steady state of circulating serum amino acids is controlled by endogenous protein stores and utilization by various tissues. Muscles produce

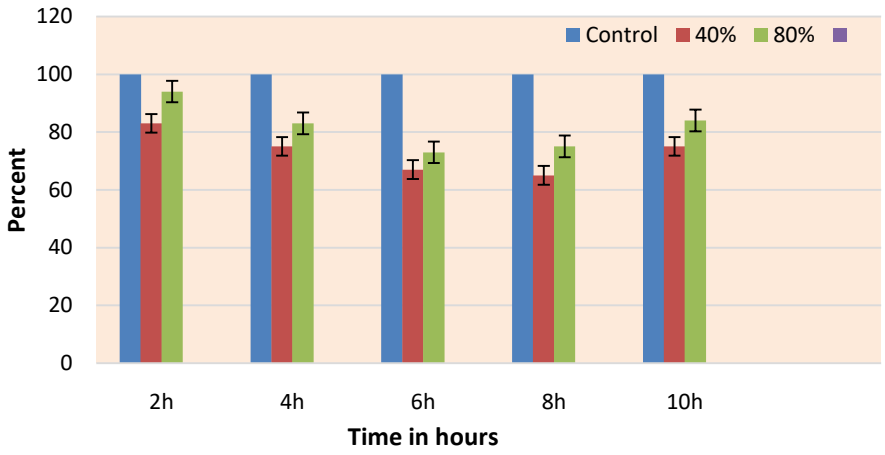

Figure 15. *In vivo* effect of 80% of LD_{50} of purified *Ropalidia marginata* venom toxins on glycogen level in atria of albino mice.

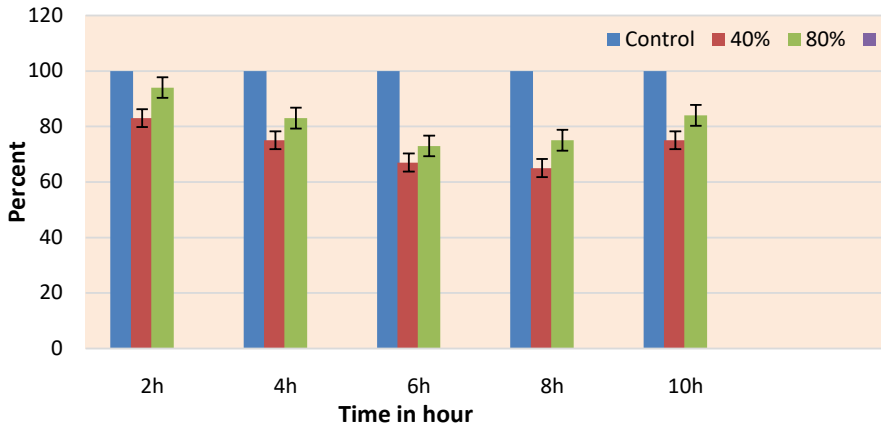

Figure 16. *In vivo* effect of 80% of LD_{50} of purified *Ropalidia marginata* venom toxins on glycogen level in ventricle of albino mice.

more than 50% of the total body supply of free amino acids, while the liver is the destroyer of excess amino acids. Thus, muscle and liver play an important role in maintaining circulating levels of amino acids [33]. This excessive release of free amino acids from the muscles or its slow uptake and removal by the liver indicates protein breakdown. Because, alkaline phosphatase activity is inhibited in gastrocnemius muscles after injection of paper wasp toxins in albino mice. It can be concluded that protein synthesis slows down in this tissue. This inhibition of protein synthesis in the gastrocnemius muscle resulted in excessive release of amino acids into the circulation, increasing serum amino acids. In addition, serum uric acid levels were found to be elevated to 138% compared to control 8 h after venom injection (**Figure 7**). It is clear that uric acid is an end product of purine metabolism, formed from xanthine by a reaction catalysed by xanthine dehydrogenase. Certain inflammatory cytokines stimulate the formation of xanthine dehydrogenase under hypoxic conditions [34]. Serum uric acid content also depends on endogenous synthesis and renal excretion. This condition also causes the kidneys of mice to malfunction and not remove uric acid effectively. The mechanism of secretion of uric acid is inhibited by many organic acids, such as lactate [35]. It occurs only in the hyperuricemia state of lactic acidosis. Uric acid is a powerful antioxidant, and its increase in serum, while its excretion decreases, increases plasma antioxidant activity. Increased plasma antioxidant activity may cause the adverse cardiovascular changes that lead to hypertension. This level of serum uric acid in a poisoned animal can fall or increase depending on the nature of the toxin found in animal poisons [36]. It is possible that the slowing of the elimination of uric acid is due to the action of the toxin component as a diuretic component. Paper wasp venom also induced hyperglycaemia in mice, resulting in increased serum glucose and cholesterol levels [37]. Similarly, serum cholesterol levels showed an increase, *i.e.*, 102%, observed at 10 hours (**Figure 8**) in experimental mice after administration of a sub-lethal dose of purified *Ropalidia marginata* venom toxins. The increase in cholesterol can be due to the formation of sphingomyelin, which explains the preferential accumulation of cholesterol in the plasma membrane. Or slow cholesterol removal or degradation of free cholesterol from membranes. This may be due to glycogenolysis and release of lipid and cholesterol molecules from membrane rupture. In addition, intracellular degradation of these molecules may also be possible. The same hyperglycaemia promote secretion of catecholamine, cortisol, thyroid and hormones, and glucagon and reduces insulin secretion. Another cause of increased serum cholesterol levels may be decreased insulin levels [38].

Paper wasps very quickly inject neurotoxins into their prey to paralyze it. They bind to and selectively bind to the membrane or membrane receptors. It causes cytotoxicity by inhibiting neuromuscular junctions and/or central nervous system and CNS function. They bind to voltage-gated sodium (Nav) and voltage-gated calcium channels (Cav), the most common targets of these toxins. Another cause of increased serum cholesterol levels may be decreased insulin

levels. The toxin of *Ropalidia marginata* also caused a significant increase in serum glucose, *i.e.*, 116% after 10 h (**Figure 10**). Cholesterol is formed in the liver and in an infected state turns into bile in the liver. Low cholesterol indicates depression, which is usually observed after poisoning in animals after a paper wasp bite. Another cause of low serum cholesterol may be the conversion of cholesterol to bile salts. The presence of the toxin also changes the level of free cholesterol, it is possible that the liver regulated plasma cholesterol, and it was also found that the liver supplies plasma with endogenous cholesterol ester and cholesterol. The decrease in serum cholesterol levels in plasma is probably due to its simultaneous accumulation in the liver. A similar decrease in serum cholesterol levels was observed in rats after administration of *Ropalidia marginata* venom [39]. Alternatively, it indicates a higher rate of oxygenation in vital tissues to combat the toxic effects of poisonous toxins. This caused a sustained increase in serum glucose and pyruvic acid levels by 116% after 6 hours (**Figure 10**). In contrast, glucose levels were found to increase by 82% in the liver, 81% in the gastrocnemius muscle at 10 hours, while glycogen levels in the heart muscle were 73% in the atrium and 67% in the ventricle at 6 hrs (**Figure 16**). The toxin can also induce glycogenolysis in stores, *i.e.*, brain, heart, kidney, adipose tissue and red blood cells. This is the main reason for the increase in blood glucose after injection of wasp venom into mice. Another reason may be the inhibition of glycogen synthesis, since glucose cannot enter the cell through transporters, the phosphorylation of glucose into glucose-6-phosphate isomerization to glucose-1-phosphate and the formation of uridine-5-diphosphate-glucose, which is the direct glucose donor for glycogen synthesis. Glycogen breakdown occurs both in the cytoplasm and within lysosomes. In cytosol, glycogen is broken down by the coordinated action of two enzymes, glycogen phosphorylase. Which releases glucose-1-phosphate by breaking T-1,4-glucosidic bonds and glycogen debranching enzyme which opens the branch sites releasing free glucose. Specifically, such a decrease in glycogen content and an increase in blood sugar may be due to toxic stress in experimental animals. Once the toxin has entered the blood, the animal experiences hypoxia and respiratory obstruction. The animal needs more oxygen for catabolism, which can only be compensated by the breakdown of blood sugar and the subsequent increase in the content of pyruvic acid. This is converted into acetyl-Co-A to produce more energy in the mitochondria. However, to maintain blood sugar, glycogenolysis is accelerated and stored glycogen is broken down. Similarly, metabolic changes in humans contaminated with synthetic venom [40]. Paper wasp venoms inhibit the secretion of insulin, an essential enzyme that regulates carbohydrate metabolism. In fact, in animals, except in the brain, insulin concentration promotes glucose self-utilization in a concentration-dependent manner. Insulin, on the other hand, stimulates the oxidation of glucose to produce more energy. However, low blood insulin levels cause slow oxidation of glucose, leading to inhibition of lipolysis [41]. Therefore, elevated glucose inhibits lipid utilization and increases its level in the blood se-

rum of poisoned mice.

5. Summary

Present paper wasp venom toxin induced alterations in levels of various biomolecules, viz. proteins, amino acids, uric acid, serum pyruvic acids, cholesterol, and glucose. For toxicity determination purified paper wasp *Ropalidia marginata* venoms it was injected in albino mice and its LD_{50} (20.6 ± 0.094 mg/kg) was determined. For performing various bioassays albino mice were injected with sublethal dose 40% and 80% of 24 hrs LD_{50} value with purified *Ropalidia marginata* venom. In treated animals wasp toxins induced stress, to get rid of this toxic stress body has utilized more stored food depots mainly carbohydrates, fats, and proteins. This is the main reason that massive glycogen was released from liver, muscles, atria and ventricle and its level was decreased significantly after two hrs of treatment. Increase in the level of free amino acids 117% compared to control after 6 hrs of venom injection. The steady state of circulating serum amino acids is controlled by endogenous protein stores and utilization by various tissues. Muscles produce more than 50% of the total body supply of free amino acids, while the liver is the destroyer of excess amino acids. Thus, muscle and liver play an important role in maintaining circulating levels of amino acids. This excessive release of free amino acids from the muscles or its slow uptake and removal by the liver indicates protein breakdown. Toxins also mobilized glycogen gastrocnemius muscles. This inhibition of protein synthesis in the gastrocnemius muscle resulted in excessive release of amino acids into the circulation, increasing serum amino acids. This level of serum uric acid in a poisoned animal can fall or increase depending on the nature of the toxin found in animal poisons [36]. It is possible that the slowing of the elimination of uric acid is due to the action of the toxin component as a diuretic component. Paper wasp venom also induced hyperglycaemia in mice, resulting in increased serum glucose and cholesterol levels. An elevated glucose level might inhibit lipid utilization and increases its level in the blood serum of toxin treated mice.

Acknowledgements

Authors are thankful to H. O. D., Department of Zoology for research facilities.

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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