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EFFECTS OF PURIFIED VENOM TOXINS FROM SPIDER *CROSSOPRIZA LYONI* ON HEMATOLOGICAL PARAMETERS IN ALBINO MICE

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ABSTRACT

In the present study venom toxins from spider venom *Crossopriza lyoni* were subjected to purify on a Sepharose CL-6B 200 column. For visualizing hematological effects sub-lethal dose of purified toxins was administered in laboratory reared albino mice. The hemoglobin level in blood was significantly increased to maximum level i.e., 118% & 128% of the control at 10 hours of treatment of 40% and 80% of 24-h LD_{50} respectively. The number of red blood cells (RBCs) was decreased to 84% and 86% while numbers of the total white blood cells (WBCs) was increased to 136% and 133% of the control after 10 hours of treatment of 40% of 24-h LD_{50} respectively. The level of mean corpuscular hemoglobin (MCH) was increased to 120% and 113% of the control at 6 hours of treatment with 40% and 80% of 24-h LD_{50} of spider venom. Low molecular weight toxic peptides ranging from 14.3-63 kDa were identified in spider venom with strong cytotoxic action that caused intra-vascular hemolysis of RBCs, leucocytes, platelets and vascular endothelium. From comparison of gel chromatographs eluted toxins peptide molecular weight was ranging from 6.2-64 kDa.

KEYWORDS: Spider venom, *Crossopriza lyoni,* gel filtration chromatography, hematological parameters.

INTRODUCTION

Spiders are predatory Arachnids which bear toxins in their sting and belong to family Arenae. Spider bites are a major problem in many Asian, African and other tropical countries

(Coddingoton *et al.,* 1991). Spider venoms represent vast sources of bioactive molecules whose diversity remains largely unknown. Indeed, only a small subset of species has been studied out of the ~43,000 extant spider species (Pallaghy *et al* 1994). These toxins possess charged amino acids which act on various ionic channels found on nerve cell membrane and cause neurotoxic and cytotoxic effect in different animal models. Spider venom peptides possess immense therapeutic value against a wide range of pathophysiological conditions cardiovascular disorders, chronic pain, inflammation and erectile dysfunction. Spider toxins induce muscle and respiratory paralysis, integumentary and show neuro-inflammatory and neuro-pathological effects in prey. Spider toxins show diverse biological effects in animals i.e. scratching, lacrimation, hypertension, salivation, sweating, agitation followed by spastic paralysis of the posterior and anterior extremities. Spider venom neurotoxins and cytolytic peptides are expressed as elongated precursor peptides, which are post-translationally processed by proteases to yield the active mature peptides. Spider venoms constitute incredibly diverse libraries of compounds, many of which are involved in prey capture and defense (Lahninger *et al*., 2017). Physiologically animal toxins are highly active block various channels and breach the normal barrier for free movement of molecules across cell membrane. Some toxins are enzymatic in nature and hydrolyze membrane phospholipids and form channels through which small molecules may pass (Fenton *et al.,* 1995). Spider venom is a major problem for farmers and gardeners. Besides this, it is a serious problem to farm animals. It has been estimated that every year millions of people died around the globe due to spider stinging. In South East Asia spider stinging is a serious problem, which can't be overlooked very easily. For quick neutralization of venom toxicity anti-venom must be provided at the right time to save the life.

Spider venom is a complex mixture bioactive compound such as enzymes, peptides, proteins, polyamines, salts and acids that show multiple biological effects in animals as well as in human. Spider venoms are complex cocktail compound of a variety of compounds, including salts, small organic molecule, peptides and proteins. It has been estimated that the potential number of unique spider venom peptides could be upwards of 12 million. Physiologically spider toxins are highly active which block various channels and breach the normal barrier for free movement of molecules across cell membrane. Some toxins are enzymatic in nature and hydrolyze membrane phospholipids and form channels through which small molecules may pass. Few toxins cause enormous hemolysis of RBCs and damage nerve cells. Venom toxins specifically act upon neurons, nicotinic acetylcholine receptors and neuromuscular

junctions (Escoubas *et al*., 2009). In the present investigation effects of purified venom toxins from spider *Crossopriza lyoni* were observed on hematological parameters in albino mice.

MATERIALS AND METHODS

(1) Spider collection and venom extraction

The Spider *Crossopriza lyoni* were collected from different building of Gorakhpur University Campus. They were immobilized by quick freezing at -20° C. The venom reservoir i.e. venom glands were taken out by chelicerae of head and homogenized in phosphate buffer saline (50mM, pH7.2) with the help of power homogenizer. The homogenate venom gland was centrifuged at 3000 g at 4°c for 5 minutes and the supernatant was used as crude venom.

(2) Solubilization of venom gland homogenate

Equal weight of venom gland of Spider *Crossopriza lyoni* were homogenized in 5.0 ml of different solubilizing buffers viz. triton X-100, phosphate buffer (50mM, pH 7.2), 10% TCA (Trichloroacetic acid), EDTA+Tris Hydroxy methyl amine and ethanol separately. Homogenate was centrifuged at 10000×g at 4°c for 5 minutes and supernatant was taken out and venom protein present in the supernatant was estimated according the method of Lowry et al (1951).

(3) PURIFICATION OF SPIDER VENOM TOXIN

(A) Preparation of gel filtration column

Gel filtration column of double cavity with sintered disc in the bottom having a height of 1 meter and 25 mm in diameter was used. The dead space inside the elution front was kept to minimum. The loading front was kept closed with a rubber cork.

(B) Selection of bead

For purification of venom proteins, Sepharose CL-6B 200 was found suitable for volumetric elution and permeation coefficient, dextran blue dye was used. The total column volume was calculated directly. The void volume was calculated and the same was re-determined with the help of the dye.

(C) Packing of the column

Slurry of Sepharose CL-6B 200 (Sigma Chemical Company, USA) was prepared in a beaker in phosphate buffer (50mM, pH 7.2) by continuous stirring. A Whatman filter paper was placed at bottom of the column above the sintered disc by pouring the distilled water. The

slurry was now poured through tubing at a normal flow rate. Stirring of the bead was continued during the column packing and packing was made without compression. For treating a high flow rate, a long piece of narrow tubing was used at the bottom of the column so as to increase the total liquid height and elution of the phosphate buffer (50mM, pH 7.2) was kept on during the entire filling. After packing, the column was continuously eluted with the phosphate buffer (50mM, pH 7.2) for 24 hours at a flow rate of 40ml/hour.

(D) Loading the sample in gel filtration column:

Buffer was eluted up to 1cm height of the loading chamber and it was drained to the gel surface very carefully. The outlet was closed with the clamp and the sample was applied with a Pasteur pipette. Sample was allowed to run 5-10 mm down inside the column and allowed to come to the surface. This was repeated with the buffer same as the volume of the sample to wash the sides of the column. When it reached to the gel surface, a continuous buffer supply was started for eluting the fractions.

(E) Elution of the venom protein through gel filtration column

Elution of the venom protein through gel filtration column was done at flow rate of 5 ml/min.

(a) Fraction collection

Eluted fractions of spider venom proteins were collected manually at a fixed time interval at a constant flow rate. Total 135 fractions were collected.

(b) Spectrophotometric observation and protein estimation of the eluted fraction

The eluted fractions were observed for the detection of venom protein at a wavelength of 280 nm. A graph was plotted between absorption at 280 nm and fraction number to show the elution pattern of spider *Crossopriza lyoni* venom protein. The protein content eluted in each fraction was determined by using the method of Lowry et al., (1951).

(c) Molecular weight determination of purified venom proteins

Range of molecular weight of different protein in the purified spider venom was determined by running the proteins of known molecular weight through Sepharose CL-6B 200 gel column as done previously. A calibration curve was drawn between Ve/Vo log M the help of calibration curve range of molecular weight of different protein in the purified spider *Crossopriza lyoni* venom was determined.

(d) Lyophilization of eluted venom protein

The eluted fractions containing venom proteins were pooled and lyophilized to a desired concentration of venom proteins. These lyophilized venom proteins will be used for their toxicity determination in animal models.

4 (A) Determination of lethality of spider *Crossopriza lyoni* venom toxins

The albino mice were injected subcutaneously with the purified venom toxins of different serial concentration and LD_{50} was determined at an interval of 24 hours. Deformities such as paralysis and neurotoxic effects were also recorded. Similarly four stage cockroach nymphs were injected with serial concentration 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 method's (Fenney *et al* 1971). The confidence limits were calculated at 95% probability levels.

4 (B) Determination of the biological toxicity of purified Spider *Crossopriza lyoni* venom toxins

For this propose one set of the laboratory reared albino mice were treated with 40% and 80% of LD_{50} of pure venom subcutaneously. Significant changes were determined in different hematological parameters such as blood hemoglobin%, packed cell volumes, plasma Hb%, red blood cells, white blood cells and mean corpuscular hemoglobin. For this purpose albino mice were injected with sub-lethal dose of the purified spider *Crossopriza lyoni* venom toxin were sacrificed after 2 hours, 4 hours, 6 hours, 8 hours and 10 hours after the injection for the collection of blood serum. To compare the effect of purified spider venom, untreated mice were given injection of the same volume of PBS buffer sacrificed and considered as control. Besides this, effects of the spider venom toxins were also observed *in vitro*.

5(A) Determination of the hematological parameters (*in vivo*)

1. Determination of blood hemoglobin

Blood hemoglobin was measured by the method of Sahli (1915). The blood was sucking in the pipette upto 0.2 marks. It was transferred in the clean hemoglobinometer tube containing small amount of N/10 HCl. The contents were allowed and shake well 10 minutes until the blood was converted into hematic acid. Now the N/10 HCl was added drop by drop into the tube and stirred continually with a glass rod. This was continued until the color of the contents exactly matched with the color of adjacent standard tubes. The end point was noted

from where the color was matched to the standard tube which shows the hemoglobin percentage.

2. Determination of packed cell volume (PCV)

In this process 1 ml blood was collected from the mice by adding EDTA as an anticoagulant. It was centrifuged at 10000 rpm for 20 minutes for the separation of plasma and blood cells. After separation, dark color settled blood cells and yellowish plasma was obtained. Packed Cell Volume (PCV) was calculated by the following formula and it was expressed in % PCV.

PCV = volume of blood corpuscles × 100/volume of blood

3. WBCs counting

The blood was sucked up to the 0.5 marks and immediately diluted the blood with PBS buffer (pH6.9) up to 11 marks and allowed it to mix WBC diluting fluids. Put a drop of blood solution on a clean counting hemocytometer and covered with the cover slip. The corpuscles were allowed to settle down and counted under the microscope. The counting was performed in the four corners of 1 $mm^{3.}$ The number of WBCs per mm^3 of the blood was calculated by the formula-

WBCs number = No. of WBCs counted × dilution × 20/ No. of squares counted mm^3.

4. RBCs counting

From each mice blood was taken out and sucked in pipette upto 0.5 ml marks by avoiding any air bubble. Immediately, Hayem's diluting fluid was sucked in the pipettes upto 10 marks and then mixed. Few drops of RBCs solution was put on the hemocytometer and covered with cover slip and allowed to settle down for 1 minute. The RBCs were counted under the microscope in 5 groups each containing 16 small squares. Thus the counting was done in 80 small squares. The total numbers of the RBCs was calculated by the formula-

RBCs number = No. of RBCs counted × dilution × 4000/No. of small squares.

5. Determination of plasma hemoglobin

For this 0.2 ml of blood was taken out by puncturing the cardiac muscles. It was quickly poured into centrifuge tube already having EDTA. Now the blood was centrifuged at 10000 rpm for 5 minutes and the upper yellowish plasma layer was taken out for the measurement of hemoglobin. Furture process was same as the blood hemoglobin.

6. Determination of mean corpuscular hemoglobin (MCH)

It was calculated by the amount of hemoglobin per liter (in gram) by the Red Blood Cells in million/mm^3.

5(B) *in vitro* study of hemolytic

In vitro hemolysis assays, blood samples were incubated with different concentration of purified *Crossopriza lyoni* spider venom toxins 5µg, 10µg, 15µg, 20µg and 25µg. Further procedure was followed similar to as *in vivo* experiments.

RESULTS

Effect of purified venom *Crossopriza lyoni* venom toxin on different hematological parameters of albino mice

In the present investigation effect o purified *Crossopriza lyoni* venom toxin on different Hematological parameters were observed in albino mice. For this purpose albino mice were injected with 40% and 80% of 24-h LD_{50} of venom toxins and bleed at 2, 4, 6, 8 and 10 hrs of the injection. Significant alterations were noted in blood hemoglobin, PCV, WBCs, RBCs Plasma hemoglobin, and MCH (Table 1 & 2).

After the injection of 40% and 80% of 24-h LD_{50} of purified *Crossopriza lyoni* venom toxins. The blood hemoglobin level was increased significantly ($p<0.05$) up to 118.58% and 128% at 10 hrs with respect to control (Table 1 & 2; Fig. A). A significant ($p<0.05$) elevation in level of PCV was observed i.e. 151.49% and 153.45% at 10 hrs of 40% and 80% of 24-h LD_{50}. (Fig. B).

The number of total WBCs was increased continuously upto 136% at 10 hrs of 40% of LD_{50} of purified *C. lyoni* venom toxins injection while it was increased significantly ($p<0.05$) upto 133% after 80% of 24-h LD_{50} at 10 hrs with respect to control (Table 1 & 2; Fig. C).

A significant ($p<0.05$) reduction in RBCs count was observed i.e. 84.20% and 86.81% at 10 hrs of treatment with 40% and 80% of 24-h LD_{50} of purified *C. lyoni* venom toxins with respect to control (Table 1 & 2; Fig. D).

Similarly, plasma Hb level was found to be increased significantly ($p<0.05$) up to 135% and 161% at 10 hrs of treatment with 40% and 80% of 24-h LD_{50} of purified *C. lyoni* venom toxins with respect to control (Table 1 & 2; Fig. E).

Mean corpuscles hemoglobin was significantly ($p<0.05$) increased up to 120% and 113% at 6 hrs of treatment with 40% and 80% of 24-h LD_{50} of purified *C. lyoni* venom toxins with respect to control. It was improved upto 103% and 97% at 10 hrs of treatment in comparison to control respectively (Table 1 & 2; Fig. F).

Table 1: *In vivo* effects of 40% and 80% of 24-h LD_{50} of purified venom *Crossopriza lyoni* toxins of on the number of RBCs, WBCs, Blood hemoglobin, MCH, PCV and plasma hemoglobin in the albino mice.

Hematology	Time in hours					
	0	2	4	6	8	10
Hemoglobin	10.26±0.016 (100)	10.56±0.012 (102)	10.98±0.012 (107)	11.09±0.016 (108)	11.78±0.016 (114)	12.12±0.030 (118)
PCV	20.00±0.124 (100)	23.00±0.124 (115)	27.50±0.20 (137)	29.50±0.12 (147)	30.00±0.21 (150)	30.19±0.17 (151)
WBC	7.311±1.24 (100)	7.89±0.07 (107)	8.34±0.016 (114)	8.78±0.016 (120)	9.56±0.03 (130)	9.98±0.01 (136)
RBC	7.31±0.016 (100)	7.19±0.016 (98.35)	6.98±8.16 (95.48)	6.88±0.016 (94.11)	6.78±0.016 (92.94)	6.20±0.016 (84.81)
Plasma Hb	0.60±1.63 (100)	0.68±1.63 (113)	0.69±8.16 (115)	0.71±0.01 (118)	0.79±4.08 (131)	0.810±4.08 (135)
MCH	8.69±3.29 (100)	8.98±4.08 (103)	9.59±3.29 (110)	10.47±0.01 (120)	9.22±0.01 (106)	9.02±0.01 (103)

Table 2: *In vivo* effects of 80% of 24-h LD_{50} of purified venom *Crossopriza lyoni* toxins of on the number of RBCs, WBCs, Blood hemoglobin, MCH, PCV and plasma hemoglobin in the albino mice.

Hematology	Time in hours					
	0	2	4	6	8	10
Hemoglobin	10.26±0.02 (100)	10.76±0.01 (104)	10.99±8.16 (107)	11.78±8.16 (114)	12.98±8.16 (126)	13.02±0.02 (128)
PCV	20.00±0.02 (100)	24.00±0.01 (120)	28.50±0.08 (142)	29.19±8.16 (145)	30.29±8.16 (151)	30.96±8.16 (153)
WBC	7.311±1.24 (100)	7.56±8.16 (103)	7.88±8.16 (107)	8.89±8.16 (121)	9.04±8.16 (123)	9.78±8.16 (133)
RBC	7.31±8.16 (100)	7.12±8.16 (97)	6.88±0.01 (94)	6.75±0.01 (92)	6.68±8.16 (91)	6.35±8.16 (86)
Plasma Hb	0.60±0.02 (100)	0.69±2.94 (115)	0.72±2.62 (120)	0.89±3.29 (148)	0.91±4.08 (151)	0.97±2.42 (161)
MCH	8.69±8.16 (100)	8.87±0.01 (102)	9.03±0.01 (103)	9.89±0.01 (113)	9.29±8.16 (106)	8.45±8.16 (97)

Values are mean ± SE of three replicates, Values in parentheses indicates percentage level with control taken as 100%, *Significant ($p<0.05$, Student t-test), *Significant ($p<0.05$, F-test), RBCs: Red Blood Cells, WBCs: White Blood Cells, MCH: Mean Corpuscular Hemoglobin, PCV: Packed Cell Volume

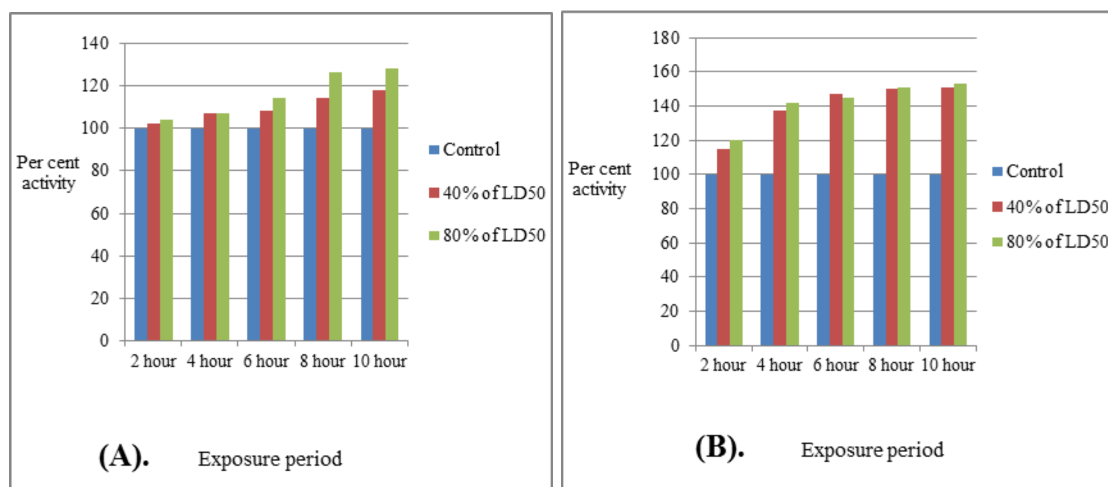
7. *In vitro* effects of the purified spider toxins venom on red blood cells

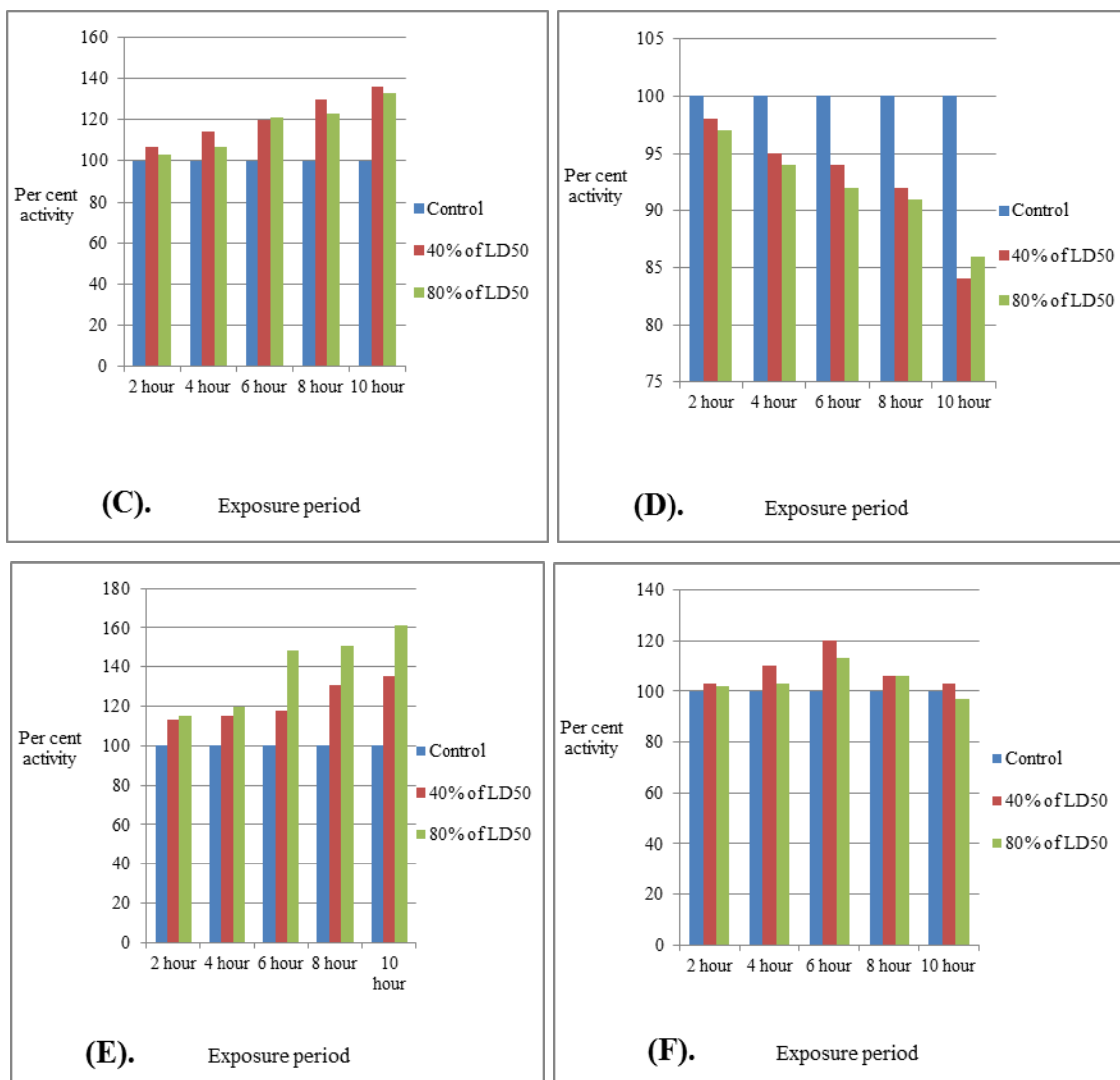
In the *in vitro* effects five concentration of purified spider *Crossopriza lyoni* venom toxins i.e., 70µg, 140µg, 210µg, 280µg and 350µg were used for hemolytic assays. *In vitro* incubation or RBCs with purified spider toxin caused dose dependent (p<0.05, f-test, student t-test) lysis of red blood cells. The percent hemolysis *in vitro* was found 10.12%, 13.45%, 25.37%, 42.8% and 62.7% at 70µg, 140µg, 210µg, 280µg and 350µg pre-incubation of purified purified spider *Crossopriza lyoni* venom respectively (Table 3).

Table 2: *In vitro* effects of different concentration of the venom *Crossopriza lyoni* toxins of on Red Blood Cells of albino mice.

S.N.	Doses in µg	% Hemolysis
1.	0 µg	0.00±0.001
2.	70 µg	10.12±0.008
3.	140 µg	13.45±0.008
4.	210 µg	25.37±0.008
5.	280 µg	42.8±0.0081
6.	350 µg	62.7±0.0082

Values are mean ± SE of three replicates, Values in parentheses indicates percentage level with control taken as 100%, *Significant (p<0.05, Student t-test), RBCs: Red Blood Cells.





***In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of spider *Crossopriza lyoni* on Hb Fig. (A), PCV Fig. (B), WBC Fig. (C), RBC Fig. (D), Plasma Hb Fig. (E), MCH Fig. (F) of albino mice.**

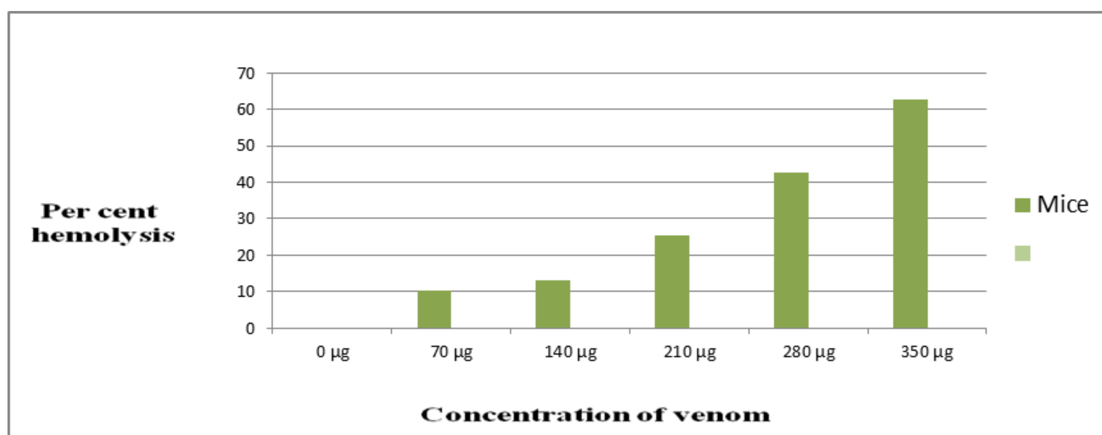


Figure 2. *In vitro* effect of different concentration of spider Crossopriza lyoni venom toxins on Red Blood Cells of albino mice.

DISCUSSION

Spider venom comprises a mixture of compound with diverse biological activities, bind a broad range of cellular targets with high affinity and selectivity and appear remarkable structural diversity. Spider venom is a rich source of bioactive compounds and contains many enzymes, proteins, polyamines and acids which show multiple biological effects in animals as well as in human. High levels of chemical diversity make spider venoms attractive subjects for chemical prospecting. More than 500 bioactive peptides from venoms of about 60 spider species with molecular weight of less than 10 kDa are characterized and divided into 20 families (Vassilevski *et al* 2009). This experimental investigation of spider *Crossopriza lyoni* venom toxin was indicate significant ($p<0.05$) deviation in certain hematological parameters Mean corpuscular hemoglobin, blood hemoglobin, Packed cell volume, White blood cells, red blood cells. A very heavy cell lysis was observed after injection of sub lethal dose of spider venom as well as blood hemoglobin concentration was increased. In venom administered albino mice rate of hemolysis become very high after 30 minutes of injection. The major reasons are release of extra corpuscular hemoglobin and its direct secretion in urine and displayed as hemoglobinuria. It is a state in which oxygen transport protein hemoglobin exceeds abnormally very high in urine. This condition is also associated with any hemolytic anemia with primarily intravascular hemolysis, in which large number of RBCs is destroyed, thereby releasing free hemoglobin into the plasma. When plasma hemoglobin concentration exceeds the hemoglobin binding capacity of kidney tubular cells get decreased. Therefore, excess free plasma hemoglobin is filtered and excreted in the urine. It results in to acute tubular necrosis and renal failure (Chugh *et al*., 1976). In the present investigation *in vitro* study of RBC hemolysis was also observed at concentration range between 70µg-350µg of *Crossopriza lyoni* venom toxin. It was dose dependent and caused potent hemolysis cause potent hemolysis (12.3%-67.7%) in albino mice. The main cause of hemolysis is membrane puncturing and formation of a transient opening by surface bound melittin. However, *in vitro* study it was come out those RBCs was the main target of *Crossopriza lyoni* venom toxins (Kalpon, 2004). Beside this, massive injection of wasp venom affects organ system and causes acute renal failure after intra-vascular hemolysis and thrombocytopenia. It also causes an intra-vascular hemolysis, pigment nephropathy and acute renal failure and tubular necrosis (Subramaniam et al., 2000; Chugh et al., 1976).

Similarly, in present study spider venom toxins caused a significant ($p<0.05$) decrease in RBCs counts 84% (Table. 1 & Fig. D) and a significant increase in number of WBCS 136%

with respect to control (Table. 1 & Fig. C). This decreases in the numbers of RBCs in blood circulation caused anemia and circulatory hypoxia in experimental animal (Ajello *et al*., 1972). The total hemoglobin content of blood was increased in albino mice treated with *Crossopriza lyoni* venom toxin. This increase may be due to hemo-concentration, which is caused by a massive release of catecholamine and angiotensin-II (Goyffon *et al.,* 1982, Radha and Vakil *et al*., 1988). Similar massive release of catecholamine, glucose, cortisol and change in thyroid hormone level after spider envenomation was reported by Goldstein (1995). Angiotensin-II produces significant decrease in the blood volume and increases in the extra vascular fluid.

In the present study, Plasma Hb and blood Hb were going to increased upto 118% and 135% respectively at 10 hrs. of spider venom toxins injection (Table. 1 & Fig. E). Similarly, MCH was also going to be increased upto 120% at 6 hrs. (Table. 1 & Fig. F), due to release of hemoglobin from ruptured erythrocytes. This increase in mean corpuscular hemoglobin is due to the hemolysis (Dacie and Lewis *et al*., 1984), the number of circulating leucocytes was found to increase 133.3% of the control after spider *Crossopriza lyoni* envenomation. Similarly, honeybee venom toxin injection caused significant reduction in red blood cells and a frequent elevation in total number of leucocytes in mice (Yousuf *et al.,* 2003). It also caused gradual increase in total number of leucocytes and hemoglobin concentration (Hussein *et al*., 2001) and possibly also effect catecholamine level (Scheuer and Stejoskins, 1969). However, leucocytosis is responsible for tissue necrosis, increased secretion of cortisol or both. Increased neutrophills counts indicate systemic inflammatory response related to cytokine release. It may be lead to induction of cytotoxic activity of toxins with hematological and immunological alterations.

Beside above change, packed cell volume was also found to be increased upto 151% at 10 hrs of spider venom toxins (Table. 1 & Fig. B). Prominently, melittin caused lysis of erythrocyte's cell membrane and also causes reduction in number of red blood cells and elevate the packed cell volume (Hider and Dotimas *et al.,* 1987). Melittin is a cationic hemolytic peptide, which bound with negatively charged phospholipids (Ghosh *et al*., 1997). Besides this, it also makes local conformational changes in the membranes (Henger and Habermann *et al.,* 1972; Walata and Gwozdzinski *et al.,* 1992; Walata and Kowalczyk *et al.,* 1992). When the number of red blood cells gets decreased due to the effect of spider venom

toxins, the adrenergic receptors stimulate the spleen to increase in venous blood flow that leads to increase in blood hemoglobin and packed cell volume (Ismail *et al*., 1992).

REFERENCES

1. Coddington, J. A., Levi, H. W. (1991). Systematics and Evolution of spider (Araneae). *Annu. Rev. Ecol. system,* 22: 565-592.
2. Pallaghy, P. K. and Norton, R. S., (1994). A common structural motif incorporating a cystein Knot and a triple stranded β sheet in toxic and inhibitory polypeptides. *Protein Sci,* 1833-1839.
3. Lehninger, A. L., Cox, M. M. and Nelson, D. L. (2000). Principal of biochemistry. *2nd ed. Worth Publishers, New York, N. K*, 633.
4. Fenton, A. W., West, P. R., Odell, G. V., Hudiburg, S. M. and Ownby, C. L. (1995). Arthropod venom citrate inhibits phospholipase A2. *J. N. Toxicon,* (33)6: 763-770.
5. Escoubas, Q., Herzig, V. and Wilson. (2009). Arachno Server: A database of protein toxins from spider. *BMC Genomics,* (10): 375.
6. Lowry, OH., Rosenbrough, NJ., Farr, AL. and Randall, RJ. (1951). Protein Measurement with Phenol Reagent. *The Journal of Biological Chemistry,* (193): 265-275.
7. Fenney, D. J. (1971). Probit analysis, (third edition) Cambridge University, London, U. K., pp. 333.
8. Sahli, H. (1915). Lehrbuch, fer Klinischen Untersuchungs-Methoden. *Leipsic, 6th edition*. 293.
9. Vassilevski, A. A. and S. A., EV, (2009). Molecular diversity of spider venom. *Biochem. Mosc*, (74): 1505-1534.
10. Chugh, K. S., Sharma, B. K., and Singhal, P. C. (1976). Acute renal failure following hornet stings. *J. Trop. Med. Hyg*, 79: 42-44.
11. Kalpon, J. (2004). What's buzzing with Africanized honeybees? *Agri. Res*, 4-8.
12. Subramaniam, C., Jain, V., Singh, M. and Kumar, L. (2000). Allergic and systemic reactions following yellow jacket stings. *Indian Pediatr*, (37): 1003-1005.
13. D Ajello, A., Zlotkin, E., Miranda, F. and Lissitzky. (1972). The effects of the scorpion venom and pure toxin on the cockroach nervous system. *Toxicon,* (10): 399-404.
14. Goyffon, M., Vachon, M. and Broglion, N. (1982). Epidemiological clinical characteristics of the scorpion Envenomation in Tunisia. *Toxicon*, (20): 337-344.

15. Radha Krishna Murthy, K., and Vakil, A. E. (1988). Elevation of plasma angiotensin level in dogs by Indian red scorpion (*Buthus tumulus*) venom and its reversal by administration of insulin and tolazoline. *Indian J. Med. Res,* (88): 376-379.
16. Goldstein, R. E., Abumrad, N. N., Wasserman, D. H. and Cherington, A. D. (1995). Effect of an acute increase in epinephrine and cortisol on carbohydrate metabolism during insulin deficiency. *Diabetes,* (44) 337-343.
17. Dacie, J. V. and Lewis, S. M. (1984). Practical hematology. 6, eds. *New York: Churchill Livinggstone,* 453.
18. Yousuf, M. I., El-demerdash, F. M., Kamel, K. I. and Al-salhen, K. S. (2003). Changes in some hematological and biochemical indices of rabbit induced by isoflavones and cypermethrine. *Toxicol,* 189(3): 223-234.
19. Hussein, A. A., Nabil, Z. I., Zalat S. M. and Rakha, M. K. (2001). Comparative study of the venom from three species of bees: effects on the heart activity and blood. *J. Nat. Toxins*, 10(4): 343-357.
20. Scheuer, J. and Stejoskins, W. A. (1969). A protective effect of increased glycogen stores in cardiac anoxia. *J. Lab. Clin. Med*, (74): 1007-1013.
21. Hider, R. C. and Dotimas, E. M. (1987). Honey bee venom. *Biochem. Biophys. Acta,* 911(3): 285-293.
22. Ghosh, A. K., Rukmini, R. and Chattopadhyay, A. (1997). Modulation of tryptophan environment in membrane-bou7nd mellitin by negatively charged phospholipids: implications in membrane organization and function. *Biochemistry*, 36(47): 14291-14305.
23. Henger, D. and Habermann, E. (1972). Bee and wasp venoms. *Science*, (177): 314-322.
24. Walata, C. and Gwozdzinski, K. (1992). Melitin induced alterations in dynamic properties of human red blood cells. Chem. Biol. Interact, 82(2): 135-149.
25. Ismail, M., Fatani, A. J., Y. and Dabeas, T. T. (1992). Experimental treatment protocols for scorpion envenomation; a review of common therapies and on effects of kallifrein-kinin inhibitors. *Toxicon*, (30): 1257-1279.