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Spider *Crossopriza lyoni* Purified Venom Toxin Induced Bio-molecular Alterations in Blood of Albino Mice

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Abstract: In the present investigation biomolecules in the blood of spider *Crossopriza lyoni* were determined in albino mice. For visualizing biomolecule effects, physiological or sub-lethal dose of purified venom toxins of spider *Crossopriza lyoni* was administered in laboratory reared albino mice. Level of serum total protein was decreased significantly ($p<0.05$) up to 78 % and 69 % at 6 h of 40% and 80% of 24-h LD_{50}, respectively as compared to control. Later on, it was slightly recovered up to 85 % and 84 % at 10 h in comparison to control. Level of serum free amino acid was increased significantly ($p<0.05$) up to 117 % at 6 h of 40% of 24-h LD_{50} of purified *Crossopriza lyoni* venom toxins while 80 % of 24-h LD_{50} caused a significant increase of 151 % at 8 h. A significant ($p<0.05$) elevation in serum uric acid was found to be 138 % and 136 % at 8 h of treatment with 40 % and 80 % of 24-h LD_{50} of purified *Crossopriza lyoni* venom toxins, respectively in comparison to control. Similarly a marginal reduction in serum cholesterol level i.e. 87 % and 88 % was found at 10 h of treatment with 40 % and 80 % of 24-h LD_{50} of purified venom toxins from *C. lyoni* in comparison to control. Serum pyruvic acid increased significantly ($p<0.05$) up to maximum 164 % at 6 h of treatment with 40 % of 24-h LD_{50} of purified *C. lyoni* venom toxins in comparison to control. Similarly, a significant ($p<0.05$) elevation in serum glucose level i.e. 152 % was noted at 10 h of treatment with 40 % of 24-h LD_{50} of purified *C. lyoni* venom toxins in comparison to control.

Keywords: Spider, *Crossopriza lyoni*, Protein, Cholesterol, Pyruvic acid, Amino acid, glucose, Uric acid, Bio-molecules, Albino mice

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Introduction

Spider venom contains a mixture of biologically active compounds with diverse biological activities. Spiders inject venom in animals that imposes muscle and respiratory paralysis and generate severe integumentary, neuro-inflammatory and neuro-pathological effects in prey. 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 patho-physiological conditions cardiovascular disorders, chronic pain, inflammation and erectile dysfunction. 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. These toxins are either acylpolyamines or polypeptides which cause irreversible paralysis in lepidopteran insects by massive transmitter release. Spider venom contains a number of insecticidal peptides that act on neuronal ion channels and receptors causes of their high specificity, potency and stability (Hu *et al.,* 2014). Spiders possess highly selective toxins and use them against insects to kill or paralyze them. Most spider venom contains multiple disulphide containing neurotoxins peptides which are pre-dominant component of venoms. A novel neurotoxin peptide was isolated from venom of the spider *Brachypelma albopilosum* having 10 kDa molecular weight (Yunhua *et al.,* 2014). Venom toxins from spider result in paralysis of muscles or stroke, muscles weakness associated with arterial hypertension, cardiac arrhythmias, myocarditis or pulmonary edema (Del *et al.,* 2013). Venom composition is highly species specific and depends on many climatic and biological factors. Spider venom includes substances of different chemical nature. The major toxic factors in most spider venom are small, disulphide rich peptides (Pineda *et al.,* 2013). Spider venoms are complex cocktail of a variety of compounds including salts, small organic molecule, peptides and proteins. These toxins have been involved in numerous aspects of cell patho-physiological effects including inflammatory response, platelet aggregation, endothelial cell hyper activation, renal disorders, and hemolysis. Spider venom contains low molecular weight inorganic and organic substances such as polyamines i.e. spermine, spermidine (Lange *et al.,* 1992). Spider venom toxin affects Na^{+} and Ca^{++} currents of ventricular myocytes because these are rich resources of cardiac channel antagonists (Haney *et al*., 2014). Spider venom toxins also show wider anti-microbial potential against communicable disease pathogens (Alanis *et al.,* 2005). Arthropods mainly spiders possess cytolytic peptides when act synergetically with neurotoxins to paralyze prey. These act on lipid membranes; and exhibit general cytotoxicity to cancer cells (Dubovskii *et al.,* 2015) Spider toxins mainly serine proteases, protease inhibitors (serpins), hyaluronidases and histamine-releasing factors showed allergen like effects in experimental animals. Enzyme toxins such as phospholipase-D family induce inflammatory response, dermonecrosis, hemolysis, thrombocytopenia and renal failure in animals (Chaves *et al*., 2017). Spider venom toxins generate necrotizing skin lesions, cell necrosis, and show systemic reactions in animals (Sapag *et al.*, 2014). Venoms also cause acute and chronic inflammatory responses in laboratory animals. Histamine inhibits vasodilation in mast cells of lungs, liver and gastric mucosa with allergic hypersensivity and inflammation. More specifically citrate present in arthropod venom inhibits phospholipase A2 activity (Fenton *et al.,* 1995). Due to toxin peptide diversity in structure and mode of action spider venom shows both local and systemic effects in animals. Therefore, chemical

treatment remains to be inappropriate solution in severe bites; hence, antibody based targeted therapy is more feasible and effective solution. Antibodies generated in response to spider venom toxins or antigens bind them efficaciously and neutralize the activity of the venom (Silva *et al.,* 2016). Spider venom is a major problem for farmers and gardeners. Spider stinging is more common in rural people and forest pockets that affect the life of people. Not only for humans, this is a serious problem to farm animals. It has been estimated that every year millions of people become morbid round 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.

In the present study sub-lethal dose of purified venom toxin of the spider *Crossopriza lyoni* was administered to albino mice. After treatment with spider toxin serum total protein, total free amino acids, serum uric acid, serum cholesterol, serum pyruvic acid and serum glucose levels were estimated at 2, 4, 6, 8 and 10 h.

Materials and Methods

(1) *Spider collection and venom extraction*

The Spider *Crossopriza lyoni* were collected from Gorakhpur University Campus. They were immobilized by quick freezing at -20 C. The venom reservoir i.e. venom glands were extirpated from chelicerae of head and homogenized in phosphate buffer saline (50 mM, pH 7.2) with the help of power homogenizer. The venom gland homogenate 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 phosphate buffer (50 mM, pH 7.2). 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 (50 mM, pH 7.2) was kept on during the entire filling. After packing, the column was continuously eluted with the phosphate buffer (50 mM, pH 7.2) for 24 hours at a flow rate of 40 ml/h.

(D) *Loading the sample in gel filtration column*

Buffer was eluted up to 1 cm 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 and with the help of calibration curve range of molecular weight of different proteins in the purified spider *Crossopriza lyoni* venom was determined.

(d) *Lyophilization of eluted venom proteins*

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

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

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 h. 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 50% 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*

Albino mice were treated with 40% and 80% of LD_{50} of pure venom subcutaneously. Total protein, total amino acids, uric acid, cholesterol, pyruvic acids, glycogen and glucose were determined in the serum at different time intervals. Albino mice injected with sub-lethal dose of the purified spider *Crossopriza lyoni* venom toxins were sacrificed after 2, 4, 6, 8 and 10 h after the injection for the collection of blood. To compare the effect of purified spider venom, untreated mice were given injection of the same volume of PBS buffer and considered as control.

(5) *Determination of blood bio-molecules*

(a) *Separation of blood serum*

Both control and treated albino mice were bled at the same time for obtaining blood. Freshly drawn blood was taken directly into a clean glass test tube without adding any coagulants. The blood was allowed to clot in cold and then centrifuged in a cooling centrifuge at 15000 rpm. Serum was collected and stored at 4 C for analysis of different biochemical parameters.

(b) *Determination of serum biochemical parameters*

(i) *Total protein*

Estimation of the total protein in the serum was carried out by Lowry's method (1915). In 0.2 ml of blood serum sample 0.3 ml of distilled water was added. Now 5.0 ml of freshly prepared alkaline copper solution (Reagent-C/analytical reagent) was added and kept for 15 minutes at room temperature. After 15 minutes 0.5 ml of Folin's reagent (Folin-Ciacalteu) was added to it. Contents were mixed well and after 15 minutes a blue color was developed which was measured at 600 nm. The volume of the total protein was expressed as µg/µl.

(ii) *Total free amino acid*

Changes in the level of free amino acids in the blood serum of albino mice were determined according to the method of Spies (1957). For this purpose 0.1 ml of blood serum was taken in glass tube. 0.1 ml of distilled water and 2 ml of the ninhydrin reagent was added to it and shacked well. The mixture was kept for 15 minutes in a boiling water bath. It was allowed to cool at room temperature and 2.0 ml of 5% ethanol was added to it. A violet color was developed that was measured at 575 nm. The value of total free amino acid was expressed as µg/µl.

(iii) *Uric acid*

Serum uric acid level was determined by the Cyanide free method of Folin (1933). In 1 ml of blood serum, 8 ml of distilled water was added then 0.5 ml of 0.66N H_2SO_4 was added to it. After 10 minutes 0.5 ml of 10% sodium tungustate solution was added and kept for 10 minutes to ensure complete precipitation. It was filtered and precipitate discarded. In 4 ml of filtered solution, 1 ml of 14% Na_2CO_3 solution and 1 ml of uric acid reagent was added and mixture was kept at room temperature for 15 minutes. Absorbance was noted at 680 nm by setting the instrument to zero density with the solution containing only water and reagent.

(iv) *Cholesterol*

Serum cholesterol level was measured according to the method of Abell *et al*. (1952). In 0.5 ml of serum, 5 ml of alcoholic KOH

solution was added. The contents were shacked well and incubated in a water bath at 37 C for 55 minutes. After cooling at room temperature added 10 ml of petroleum ether and mixed well. Then added 5 ml of water and shacked vigorously for 1 min. The content mixtures were centrifuged at slow speed (1200 rpm) until two clear layers of petroleum ether and water was obtained. Now 5 ml of aliquot petroleum ether was transferred to a dry test tube and placed in a water bath at 60 C for evaporation of the solvent, a gentle stream of air was blown over the solvent. Then, 6 ml of Lieberman-Burchard reagent was added in each test tube. Test tubes were shacked and returned to water bath. After 30 minutes absorbance was determined at 620 nm.

Standard cholesterol solution was prepared by dissolving 100 mg dry cholesterol in sufficient absolute alcohol to make volume up to 250 ml.

(v) *Pyruvic acid*

The level of pyruvic acid was determined according to the method of Freidman and Haugen (1943). For this purpose blood serum was de-proteinized with 5% TCA containing 0.10% silver sulphate and centrifuged at 10000 rpm for 10 minutes. Then 1 ml of 2,4-Di-nitrophenyl hydrazine was added to 0.10 ml de-proteinized serum. It was allowed to react at room temperature for 15 minutes. Same procedure was carried out with dilute pyruvic acid standard solution. Now 3 ml of xylene was added, air was passed and left the mixture for 2 minutes. After setting reaction mixture, the lower layer was discarded by using pipette. Then 6 ml of 10% sodium carbonate was added and mixed for 2 minutes again by bubbling the air through the mixture and allowed to settle. 5.0 ml of the aqueous layer was taken out in test tubes and 5.0 ml of 1.5 N NaOH solutions was added to it. It was mixed thoroughly and left for 10 minutes. Absorbance was read at the 520 nm after setting the instrument at zero absorbance with blank containing 5.0 ml of 10% sodium carbonate and 5.0 ml of the 1.5 N NaOH. The serum pyruvate was measured in terms of mg/100 ml of the blood serum.

(vi) *Glucose*

Serum glucose level was measured according to the method of Mendel *et al.* (1954). For this purpose 0.5 ml of the blood serum was deprotienized by 5% TCA containing 0.1% silver sulphate. The mixture was centrifuged at 10000 rpm for 10 minutes. In this 0.5 ml of the deprotienized supernatant and 4.5 ml of H_2SO_4 was added and mixed thoroughly. Contents were boiled in water bath for 6 minutes and the mixture was kept at room temperature for cooling. The pink color was developed which was read at the 520 nm. The blank contains only 0.5 ml of 5% TCA containing 0.1% silver sulphate and 4.5 ml of H_2SO_4. The glucose level was expressed as mg/100 ml of blood serum.

Standard glucose solution was prepared by mixing of 500 mg of glucose power in 100 ml of distilled water. So the concentration of glucose/glycogen content was 5 µg/µl.

Results

Effects of purified Crossopriza lyoni on different bio-molecule in mice serum

Effects of purified *Crossopriza lyoni* venom toxins were evaluated on some biomolecules

Table 1: *In vivo* effects of 40% of 24-h LD_{50} of purified venom toxins of *Crossopriza lyoni* on the bio-molecules such as total protein, free amino acids, glucose, pyruvic acid, uric acid, cholesterol and glucose in the albino mice

Parameters	Time in hours					
	0	2	4	6	8	10
Protein	0.75±1.24 (100.0)	0.71±1.63 (94)	0.61±1.63* (81)	0.59±1.63 (78)	0.62±2.05 (82)	0.64±1.63 (85)
Free amino acid	0.092±8.16 (100.0)	0.095±0.01 (103)	0.099±1.63 (107)	0.108±8.16* (117)	0.093±8.16 (101)	0.084±8.16 (91)
Uric acid	0.282±8.16 (100.0)	0.295±1.24 (104)	0.305±8.16 (108)	0.375±1.24* (132)	0.390±8.16* (138)	0.370±1.63 (131)
Cholesterol	0.385±8.16 (100.0)	0.396±8.16 (103.3)	0.410±1.63 (107.0)	0.425±1.24 (111.6)	0.401±2.86* (118.0)	0.380±1.63* (125.0)
Pyruvic acid	0.25±1.63 (100.0)	0.29±1.24 (116)	0.35±1.69* (140)	0.41±8.16* (164)	0.36±0.36* (144)	0.31±0.31 (124)
Glucose	0.75±1.63 (100.0)	0.85±1.63 (113)	0.89±1.63 (118)	0.98±1.63* (130)	0.102±8.16* (136)	0.114±4.71* (152)

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)

Table 2. *In vivo* effects of 80% of 24-h LD_{50} of purified venom toxins of *Crossopriza lyoni* on the bio-molecules such as total protein, free amino acids, glucose, pyruvic acid, uric acid, cholesterol and glucose in the albino mice

Parameters	Time in hours					
	0	2	4	6	8	10
Protein	0.75±8.16 (100.0)	0.69±2.05 (92)	0.61±1.24 (81)	0.52±8.16* (69)	0.59±1.24 (78)	0.63±1.24 (84)
Free amino acid	0.092±8.16 (100.0)	0.122±8.16 (132)	0.129±8.16 (140)	0.131±1.24* (142)	0.139±8.16* (151)	0.126±8.16 (136)
Uric acid	0.282±1.24 (100.0)	0.298±8.16 (105)	0.303±8.16 (107)	0.351±8.16* (124)	0.385±1.24* (136)	0.210±1.63 (74)
Cholesterol	0.385±8.16 (100.0)	0.399±8.16 (103)	0.426±8.16 (110)	0.440±2.49* (114)	0.375±8.16 (97)	0.360±1.63 (93)
Pyruvic acid	0.25±1.63 (100.0)	0.28±1.63 (112)	0.35±1.63 (140)	0.39±1.63* (156)	0.41±1.24* (164)	0.40±6.18 (160)
Glucose	0.75±8.16 (100.0)	0.95±8.16 (126)	0.98±8.16 (130)	0.98±8.16 (130)	0.104±8.16* (138)	0.109±8.16* (145)

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)

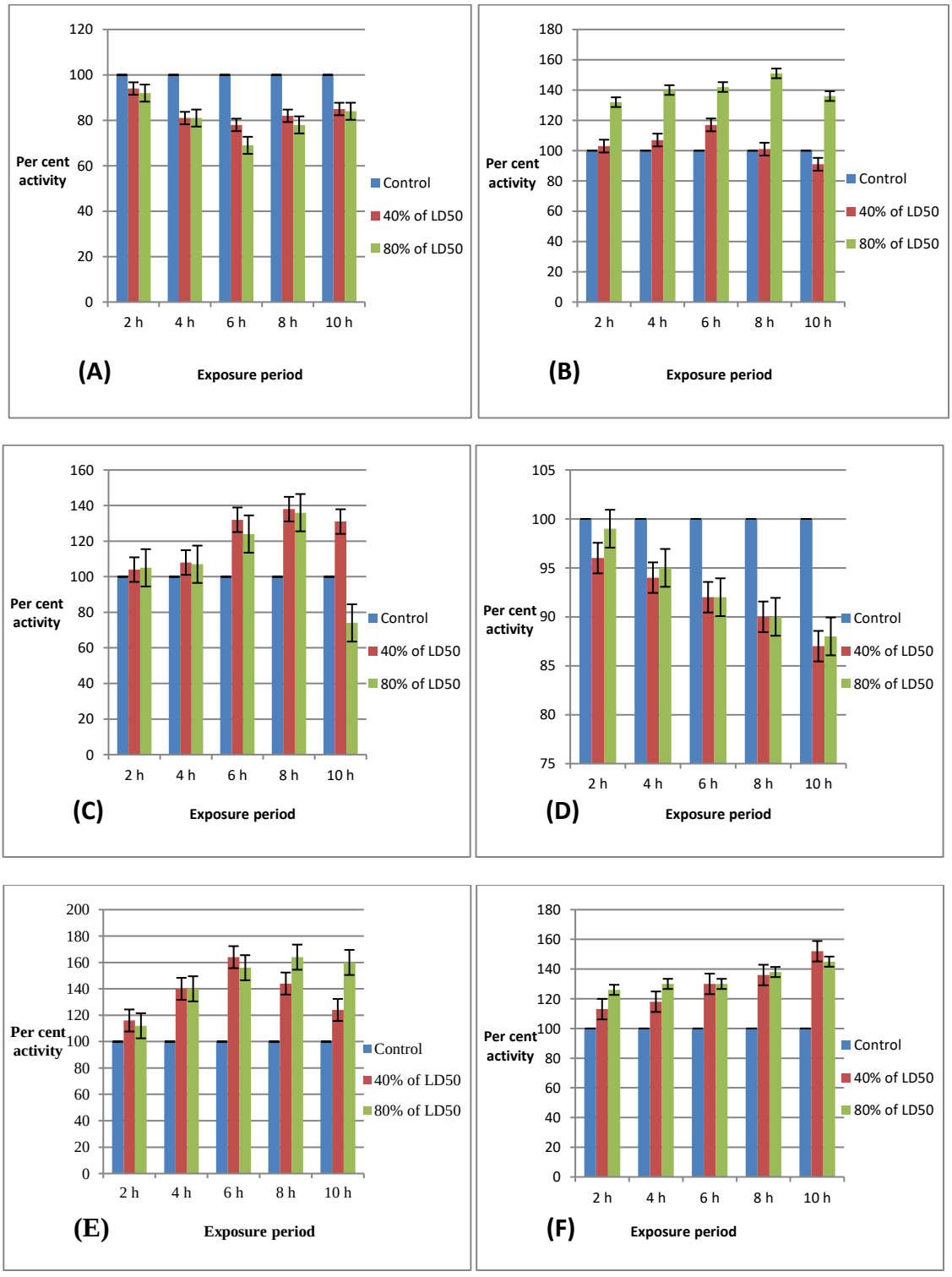

Fig. 1: *In vivo* effect of 40% and 80% of 24-h LD_{50} of purified venom toxins of spider *Crossopriza lyoni* on serum protein (A), amino acid (B), uric acid (C), cholesterol (D), pyruvic acid (E) and glucose (F) of albino mice.

in blood serum of albino mice. The change in the level of certain bio-macromolecule i.e. glucose, total protein, free amino acid, uric acid, pyruvic acid and cholesterol were measured after intra-peritoneal injection 40% and 80% of 24-h LD_{50} of purified *Crossopriza lyoni* venom toxins.

Content of serum total protein decreased significantly ($p<0.05$) up to 78% and 69% at 6 h following treatment with 40% and 80% of 24-h LD_{50} of venom toxin with respect to control. Later on it slightly recovered up to 85% and 84% at 10 h in comparison to control (Table 1, 2; Fig. 1 A).

Serum free amino acid content increased significantly ($p<0.05$) up to 117% at 6 h after exposure to 40% of 24-h LD_{50} of purified *Crossopriza lyoni* venom toxins while after treatment with 80% of 24-h LD_{50} there was a significant increase (151%) at 8 h. Further, it was recovered 91% and 136% at 10 h of the treatment in comparison to control, respectively (Table 1, 2; Fig. 1B).

A significant ($p<0.05$) elevation in serum uric acid was noticed i.e. 138% and 136% at 8 h of treatment with 40% and 80% of 24-h LD_{50} of purified *C. lyoni* venom toxins in comparison to control, respectively (Table 1, 2; Fig. 1C).

A reduction in serum cholesterol level i.e. 87% and 88% was found at 10 h after treatment with 40% and 80% of 24-h LD_{50} of purified venom toxins from *C. lyoni* in comparison to control, respectively (Table 1, 2; Fig. 1D).

Serum pyruvic acid increased significantly ($p<0.05$) up to maximum 164% at 6 h after treatment with 40% of 24-h LD_{50} of purified *C. lyoni* venom toxins in comparison to control. However, it became almost normal (124%) at 10 h after treatment with same dose (Table 1, 2; Fig. 1E).

A significant ($p<0.05$) elevation in serum glucose level i.e. 152% was noted at 10 h after treatment with 40% of 24-h LD_{50} of purified *C. lyoni* venom toxins in comparison to control (Table 1, 2; Fig. 1F).

Discussion

Crossopriza lyoni venom envenomation caused malfunctioning of kidney which resulted in elevation in serum creatinine phosphokinase-MM isoenzyme levels suggesting rhabdomyolysis (Chugh *et al*., 1976). A low total protein level showed a liver disorder or a kidney disorder, or a disorder in which protein is not digested or absorbed properly. Contrary to this, in the present study level of serum free amino acids was found elevated 132% at 10 h after venom injection. Changes in *serum free amino acid level* may occur in patients with spleen deficiency syndrome or due to muscle performance decrements. It suggests that wasp venom toxins also exhibit strong proteolytic activities that make reduction in serum total protein (Lima *et al.,* 2000; Sousa *et al.,* 2001). Besides *Crossopriza lyoni*, other insects such as ant *Eciton burchelli* (Schmidt *et al.,* 1985) and snake venom have also shown strong proteolytic activities (Gutierrez and Lomonate, 1989; Koh *et al*., 2001). Further, it may be due to presence of protease enzymes in spider venom, which strongly act on protein and peptides and make their conversion into free amino acids (Lima *et al*., 2000). Probably this conversion elevates the level of free amino acid in the serum of albino mice. Similarly, the proteinase isolated from *Bothrops leucurus* (white tailed jararaca) snake venom has also

shown proteolytic and fibrinolytic activity (Bello *et al*., 2006). Besides this, few non-insect poisons also show a significant reduction in the concentration of protein (Krauze *et al*., 2007). The decline in total protein level with increase in transaminase activity suggests the mobilization of free amino acids during the venom induced stress condition to meet the energy demands (Zeba and Khan *et al*., 1995). Spider venom toxin also causes change in bio-molecule in albino mice after envenomation. However, in the present study level of serum total protein was found to be reduced upto 78% at 6 h in albino mice provided with sub lethal dose of purified *Crossopriza lyoni* venom toxins.

Contrary to this level of serum free amino acid was found to be increased significantly upto 117% at 6 h of venom injection with respect to control. It suggests that spider venom toxins also having strong proteolytic activity which reduced serum total protein level (Lima *et al.*, 2000). The steady state concentration of circulating serum amino acid is under the control of endogenous protein stores and utilization by various tissues. Muscle generates greater than 50% of total body pool of free amino acids while the liver is the site of the disposal of excess amino acids. Thus muscle and liver play major role in maintaining circulating amino acid level (Victor *et al*., 1996). The elevated amino acid level in serum is probably the release of excess free amino acid from muscle or its slow uptake and removal by the liver. Alkaline phosphatase activity is inhibited in gastrocnemius muscles after spider envenomation. It can be inferred that in this tissue protein synthesis is retarded. This inhibition in protein synthesis in the gastrocnimius muscle causes it to release excess amino acid in the circulation, thereby, increasing amino acid level in the serum.

Moreover, serum uric acid level was also increased up to 138% at 8 h after venom injection. It is evident that uric acid is the end product of purine metabolism, formed from xanthine via a reaction catalyzed by xanthine dehydrogenase. Formation of xanthine dehydrogenase was induced by certain inflammatory cytokines in hypoxia condition (Meneshian and Bulkely, 2002). Serum uric acid depends on endogenous synthesis (Leyva *et al*., 1998) and renal excretion (Quinines *et al*., 1995). This condition also arise dysfunction of mice kidneys, and these don't eliminate uric acid efficiently. The secretory mechanism of uric acid is inhibited by a number of organic acids like lactate. This accounts for the hyperurecimia in lactate acidosis. Uric acid is the powerful anti-oxidants and its elevation in serum by reduction in its excretion entails increase in plasma anti-oxidant activity. Increased anti-oxidant activity of plasma is possibly responsible for the unfavorable changes in the heart and the vessels that results in hypertension. In contrast to increased serum uric acid level in *Heterometrus fastigiousus* envenomated animal, its level in serum was reported to decrease in experimental mice after *Leiurus quinquestriatus and Palamneus gravimanus* envenomation (Omran and Abdel-Rahman, 1992; More *et al.,* 2004). There is a possibility that a slow-down in the removal of uric acid is due to effect of toxin component as a diuretic compound. Similarly, in experimental mice treated with sub-lethal dose of the purified *Crossopriza lyoni* venom toxins, a marginal elevation i.e. 125% in serum cholesterol level was observed at 10 h. Spider venom also caused hyperglycemia in mice

leading to elevation in serum glucose and cholesterol level (Daisley, 1988). The reason behind elevation in the level of cholesterol may be elevated sphingomyelin content propose to account for preferential accumulation of cholesterol in the plasma membrane, or slow rate of cholesterol clearance or disruption of free cholesterol from membranes. It may be due to glycogenolysis and liberation of lipid and cholesterol molecules from membrane disruption. Besides this, intracellular disruption of these molecules may also be possible. Another reason of elevation in serum cholesterol may be due to lowering of insulin level (Scheuer *et al*., 1969).

Membrane-active peptides (MPs) are a class of bio-molecules that play an important role in the existence of certain organisms and their communities. For example, antimicrobial membrane-active peptides (AMPs), which selectively act on the membranes of various cells, are among the main effectors in the "innate immunity" system, which is the earliest defense system of eukaryotes (Zasloff *et al.,* 2002). During the predation, spiders inject neurotoxins which is able to cause paralysis of their prey due to the blocking actions at the neuromuscular junctions and/or at the central nervous system (CNS); generally the voltage-gated sodium (Nav) and voltage-gated calcium (Cav) channels constitute the most common targets of these toxins (Hagiwara *et al*, 1991; Glenn and King, 2007).

Another reason of elevation in serum cholesterol may be due to lowering of insulin level. *Crossopriza lyoni* venom toxin also caused significant increase in serum glucose level i.e. 152% at 10 h. Cholesterol is produced in liver and converted to bile in the liver. Low cholesterol is the symptoms of depression and hyperthyroidism which have been reported to occur after spider *C. lyoni* stinging the patients and animals (Radha Krishna Murthy and Zare, 1998). Other cause of low serum cholesterol may be conversion of cholesterol to bile salts (Guyton, 1991). Since the lipoprotein is associated with the cholesterol aster and free cholesterol, it is possible that liver might have regulated the plasma cholesterol. Further it is established that liver supplies endogenous cholesterol ester and cholesterol to plasma (Rodrigues *et al*., 1986). Reduction in serum cholesterol occurs probably due to accumulation in liver (Mandal and Lahiri, 1989). Omran and Abdel-Rahman (1992) reported decrease level of serum cholesterol in rat envenomated by *Leiurus quinquestriatus* venom.More *et al*. (2004) have also reported that intra-muscular envenomation of *Palamaneus gravimanus* venom in rat caused significant decrease in serum cholesterol. It indicates higher oxidation rate which cause continuous increase in serum glucose level. Therefore, pyruvic acid level was found to be increased up to 164% at 6 h. This may be due to massive utilization of glucose for removing the toxic stress. However, hyperglycemia increases the secretion of catecholamines, glucagon, cortisol, thyroid hormones and reduced insulin secretion (Scheuer *et al.,* 1969). Similar increase in blood sugar was also reported in dogs following envenomation by scorpion venom *Mesobuthus mulusconcanesis* (Murthy and Haghanazari, 1999).

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