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HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN LIONFISH *(PTEROIS RUSSELII)* VENOM TREATED SWISS ALBINO MICE

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ABSTRACT

The effect of lionfish *(Pterois russelii)* venom was examined on certain biochemical and haematological parameters in experimental mice. Crude venom was extracted from the spines of lionfish. Mice blood (0.5ml) was collected before and after injection of the venom. The haematological parameters were determined by using Haemocytometer and biochemical markers were analyzed by Auto analyzer using diagnostic kits. Haematological changes were drastically decreased after 6 hours of injection of 3.75µg/kg b.w. (10% of the LD_{50} value) of the venom and were reduced till 24 hours and start to recover after 48 hours. Biochemical markers AST, ALT, ALP, LDH and CPK were increased after 6 hours of venom administration and were significantly high till 48 hours. Similarly, blood glucose and urea levels were also significantly increased after venom injection. On the other hand, no significant difference was observed in the total protein, creatinine and TG levels after venom injection. The cholesterol levels remain unchanged throughout the experimental period. This experimental result suggests that the venom has short term reversible effect in a mouse model at sub lethal dose.

KEY WORDS: **Lionfish (Pterois russelii), Mice, Haematological, Biochemical markers.**

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INTRODUCTION

Marine environment has always been considered as rich sources of both biological and chemical diversity. Marine organisms are the main sources of structurally diverse bioactive compounds. The great deal of interest has been expressed regarding marine derived bioactive peptides because of their numerous health related beneficial effects[1]. Marine organisms represent an enormous, essentially unexploited resource of natural products for the discovery of compounds[2] with pharmacological properties like anticarcinogenic, antibiotic, growth promoting or inhibiting, haemolytic, analgesic, antispasmodic, hypertensive and hypertensive agent[3] and even anti-HIV agents[4]. Besides these pharmacological agents, marine environment produces nutritional supplements, cosmetics, agro-chemicals, molecular probes, enzymes and fine products that have a potential multi-billion dollar market value[5]. Although bio-prospecting of marine organisms had yielded many bioactive peptides, pharmacologists have great passion for getting venom associated peptides and to know their role in human metabolism. Since the medicinal value of venoms has been known from ancient times, venom toxins have been increasingly used as pharmacological tools and as prototypes for drug development. Venoms represent a huge and an essentially unexplored reservoir of bioactive compounds that may cure disease conditions which do not respond to currently available therapeutics[6]. Fish venoms are focused as a potential source of pharmacological agents and physiological tools that have evolved to target vital processes in human that appears to have more selectivity than many other drugs. Lionfish are venomous with their spines contains apocrine type of venom glands. Each spine of the lionfish (except caudal spines) is venomous including 13 dorsal spines, three anal spines and two pelvic spines. The spines are encased in an integumentary sheath or skin and contain two grooves of glandular epithelium that comprises the venom producing tissue. Lionfish envenomation occurs when the spines integumentary sheath is depressed as it enters the victim. This process tears the glandular tissue allowing the venom to diffuse in to the puncture wound[7]. Lionfish venom has been found to cause cardiovascular, neuromuscular and cytolytic effects ranging from mild reactions such as swelling to extreme pain and paralysis in upper and lower extremities[8]. The toxin of lionfish venom contains acetylcholine and neurotoxin that affects neuromuscular transmission[9]. Antivenom of the related stonefish *(Synanceia spp.)* is highly effective in neutralizing lionfish venom activity [10] and [11]. Biological properties of venom from terrestrial animal have been extensively investigated, while scarce research has been undertaken on fish venom. Most of the proteinaceous venoms showing marked curative properties are from highly toxic species (snake, scorpion, etc) and their high toxicity hinders clinical trials. Hence, less toxic species like fish represent a valuable source of pharmacological compounds that may lead compounds for new drugs. The present study was designed to investigate Haematological and biochemical changes in lionfish (*P. russelii*) venom treated mice.

MATERIALS AND METHODS

Venom preparation

Live specimens of the venomous lionfish (*P. russelii)* were collected from Mandapam coast and brought to the laboratory. Crude venom was extracted as described by church and Hodgson[11]. The fishes were killed (by cooling) and the venomous spines were removed and stored in 10% glycerol solution at -80^0 C. When required, the spines were thawed and grounded in a chilled mortar and pestle with 10% glycerol solution. The suspension was kept in a magnetic stirrer (overnight) at 2^0 C and centrifuged at 7000g for 15 minutes in a refrigerated centrifuge. The supernatant was removed and the protein was estimated by the method of Lowry[12] and the concentration was adjusted to 1.0 mg/ml, aliquot and stored at -20^0 C until to use.

Determination of LD_{50}

The LD_{50} value was determined in mice by following the OECD guidelines No.425 (up and down method) and the values are calculated by the method of Litchfield and Wilcoxon[13].

Experimental animal

Adult male Swiss albino mice *(Mus musculus)* of 10 to 12 weeks old (22 ± 2 g) were obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. The animals were maintained under controlled conditions of temperature (23 ± 2^o C), humidity (50 ± 5%), and light (10 and 14 h of light and dark cycles, respectively) and were fed with commercial standard pellet and provided water *ad libitum*. Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, Annamalai University (Registration Number: 953/2012/CPCSEA) and the animals were cared in accordance with the “Guide for the care and use of laboratory animals” and “Committee for the purpose of control and supervision on experimental animals.”

Blood sample collection

The lionfish venom at a concentration of 10% of LD_{50} - sub lethal dose was administered to twelve animals through single intraperitoneal injection. After 6, 12, 24 and 48 hours, 0.5 ml of blood sample was collected from the tail vein of six animals in vials containing EDTA solution (2.7 g/100 ml) as an anticoagulant for hematological studies and 0.5ml of blood from the remaining six animals were collected without anticoagulant and serum was separated from them and used for the enzyme analysis.

Haematological studies

From the collected blood samples serum was separated by centrifugation at 3000g for 30min and analyzed for the following various hematological parameters. Haemoglobin content (Hb), red blood cell (RBC), white blood cell (WBC), haematocrit value (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), platelet count were determined by using haemocytometer.

Biochemical studies

Serum was separated by centrifugation at 3000g for 30 min and used for the determination of various enzymes present in it. Serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH), creatinine phosphokinase (CK), cholesterol, blood glucose, triglycerides (TG), urea and total protein was estimated in an auto analyzer (Robonik (India) Pvt. Ltd.) by using diagnostic kits, [Robonik, Prietest™, Clinical Chemistry Reagent (IVD Test Kits)].

Statistical analyses

The experimental data were expressed as means ± Standard Deviations. Differences in means were estimated by using SPSS 16.0. One-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) were also done. In all cases statistical significance is indicated by $P < 0.05$.

RESULTS

LD_{50} Determination

The crude venom dose which killed 50% of the tested animals is known as LD_{50}. It was 37.5 µg/ kg body weight for Swiss albino mice after intraperitoneal injection of lionfish *(P. russelii)* venom.

Biochemical studies

Haematological profile

The changes in the levels of blood parameters are given in Table 1 and Fig.1. Red blood cell, White blood cell, haemoglobin, haematocrit value, mean corpuscular haemoglobin, mean corpuscular volume and platelet counts were drastically decreased after 6 hours of injection of 3.75 µg/kg b.w (10 % of the LD_{50} value) concentration of the venom and were found to be reduced till 24 hours and started to recover after 48 hours.

Table 1

Effect of lionfish venom on the levels of haematological parameters of control and treated mice.

Parameters	Control	Six hours post injection	Twelve hours post injection	Twenty four hours post injection	Forty eighty hours post injection
WBC ($x10^3$/µl)	7.8±0.58	8.0±0.61*	7.2±0.56*	6.7±0.53*	7.3±0.42*
RBC ($x10^3$/µl)	4.7±0.43	5.3±0.41	4.2±0.31*	3.1±0.18*	4.6±0.19*
Hb (g/dl)	12.8±0.76	10.5±0.90	10.3±0.83*	9.7±0.81*	9.9±0.74
HCT (%)	43.1±3.34	41.2±3.11*	35.9±2.9*	33.7±2.71*	37.2±2.51*
MCV(fl)	95.6±6.31	93.1±6.14	91.8±6.01*	87.5±6.18*	88.3±6.57
MCH (pg)	27.1±2.5	26.8±2.12	25.8±2.15*	25.7±2.03*	24.2±0.98*
PLT ($x10^3$/µl)	349.1±22.8	266.4±20.22*	217.8±14.78*	202.6±14.51*	289.4±12.69*

(Values are mean ±SD, n=6, * P < 0.05, control and venom treated animal)

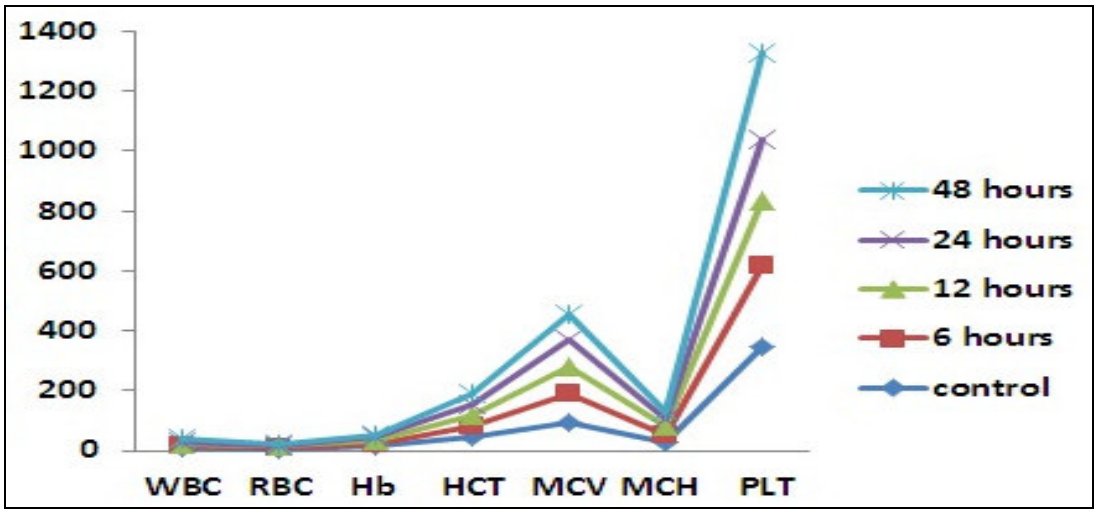

Figure 1

Effect of lionfish venom on the levels of haematological parameters of control and treated mice.

Biochemical markers

The changes in the levels of serum biochemical markers are shown in Table - 2 and Fig. 2. The serum markers AST, ALT, ALP, LDH and CPK were increased after 6 hours of venom administration and were significantly high until 48 hours. Similarly, blood glucose and urea levels were also significantly increased after venom injection. On the other hand, no significant difference was observed in the total protein, creatinine and TG levels after venom injection. The cholesterol level was remained unchanged throughout the experimental period.

Table 2
Effect of lionfish venom on the levels of serum biochemical markers of control and treated mice

Parameters	Control	Six hours post injection	Twelve hours post injection	Twenty four hours post injection	Forty eighty hours post injection
AST (1v/L)	11.2±0.78	11.9±0.6*	13.5±0.7*	16.8±0.2*	12.2±0.3*
ALT (1v/L)	5.2±0.2	5.8±0.1*	6.8±0.5*	9.1±0.1*	6.9±0.2*
ALP (1v/L)	30±0.28	34±0.1*	37.5±0.2*	44.2±0.3*	34.1±0.5*
LDH (1v/L)	70±7.2	78±0.6*	88±8.5*	107±10.2*	84±10.0*
CPK (1v/L)	12.1±1.24	16±0.2*	17±1.6*	21±0.1*	14±0.2*
Blood sugar (mg/dl)	82.4±0.5	92±0.7*	96±0.6*	101±10.2*	87.8±7.6*
Urea (mg/dl)	20.2±2.1	61.2±4.2*	39±5.5*	26±2.9*	25±1.3*
Protein (mg/dl)	5.9±0.2	5.8±0.6	7.1±0.5	6.1±0.3	6.4±0.1
Creatinine (mg/dl)	0.7±0.4	1.1±0.2	0.7±0.04	0.7±0.04	0.7±0.04
TG (mg/dl)	154±14.4	162±15.7	161±14.0	155±14.8	153±13.7
Cholesterol (mg/dl)	71±6.0	75±6.2	74±7.11	73±6.1	73±5.2

(Values are mean ±SD, n=6, * P < 0.05, control and venom treated animal)

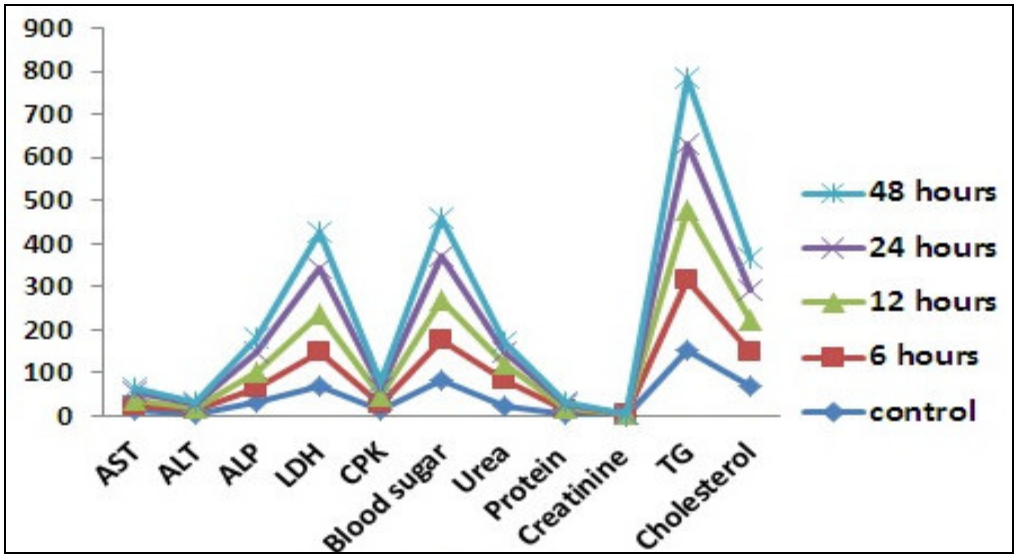

Figure 2
Effect of lionfish venom on the levels of serum biochemical markers of control and treated mice.

DISCUSSION

Toxic venomous proteins are having a number of adverse effects such as immobilizing, paralyzing, killing, and liquefying activity on pray organisms. The venom proteins may act synergistically by enhancing the activity or spreading of toxins[14]. Discoveries of toxins from venoms, for the most part from marine resources that are racing ahead because of their extremely complex and notable action on various mammalian physiological systems[15]. The present investigation was carried out to determine the effect of lionfish (*P. russelii*) venom on haematological and biochemical levels in mice. Haematological and biochemical studies are an important tool for health evaluation and their interpretations to know the status of physiological functions of various organs. Lionfish (*P. russelii)* venom in mice produced a spectrum of activity, which has a number of biochemical and physiological properties. The LD_{50} value of the venom was 37.5 µg/kg body weight in mice, a value that is comparable to the LD_{50} value of venoms of other members of the family Scorpaenidae such as, *S. gutla* (LD_{50} 260µg/kg), *S. horrida* (30µg/kg), *S. verrcosa* (125µg/kg) and *S. plumieri* (280µg/kg)[16]. Several works dealing with the effects of venoms in blood cells, bone marrow cells and also cells from muscle, liver, kidney and skin, showed varying results, depending on the experimental concentrations, exposure time, site of injection, and type of toxin[17 and 18].

Hematological and Biochemical changes

Administration of 3.75µg/kg body weight (10% of LD_{50} sub lethal dose) of the venom on male Swiss albino mice showed the irreversible biochemical changes that occur during the administration. The results of blood analysis show that *P. russelii* venom produces significant alteration in the haematological parameters. Reduction in Hb, HCT, MCV, MCH and PLT level was related to the decreased blood cell count and is leading to increased haemolysis. Similar results were observed by using venom of various species like conus, snake, and scorpion[19]. Anil and Priyanka[20] reported that lethal and sub lethal doses of catfish venom are capable of stimulating stress reaction and cause the significant decrease in RBC count, WBC count and Haemoglobin percentage which are needed for the normal physiology of animals. Saminathan[21] have suggested that proteolytic enzymes present in venom are the prime cause for lysis of blood cell membrane. They digest the integral protein and there by weaken certain membrane permeability, increased osmotic fragility and

haemolysis. Enzymes are present throughout the body and their measurement can provide valuable diagnostic information. Among all organs, the liver is considered to be the main target organ for drug activations, detoxification and other metabolic reactions. The liver is a major organ for the production of serum proteins and its total level in the blood is considered as main liver function test. Shaban and Hafez[22] observed the activity of some liver marker enzymes and were measured in the plasma of venom injected rats to assess their impact on vital organs. The increased level of AST, ALT, ALP and LDH reflects the functional status of the affected organs. The observed increase in the activities of ALT could be attributed to the hepatocellular injury and elevation in AST and CPK may result from conditions causing injury to cardiac muscle. Similar association between biochemical finding and histological changes were reported by Nagwa M. El-Sawi[23]. It shows that, as the lethality increase the mobilization of enzymes from damaged tissue into circulation also increased[24]. Carlos[25] reported that snake venom induced the release of LDH and transaminases (ALT and AST) indicating tissue and liver abnormalities. Omaran and Rahman[26] reported that lethal and sub lethal doses of venom are capable of stimulating stress reaction, which causes the release of cortisol and catecholamine's in circulation, which in turn cause several damage in many vital organs and is proportional to the dose of venom and elapsed time. The levels of blood glucose and urea were significantly increased after administration of the *P. russelii* venom. The severe hyperglycemia may be due to the effect of an increase in the catecholamine level, which causes glycogenolysis and mobilizes the glycogen from liver[27]. The increased level of serum urea in lionfish venom treated mice indicates the evidence of renal damage and the finding are in line with those observed by Saminathan[21].

CONCLUSION

The measurements of biochemical parameters following lionfish (*P. russelii)* crude venom injection, clearly demonstrate the damage of vital organs, especially liver, kidney, bone marrow and muscles. Such damages in vital organs are remaining at least for 6 hours after envenomation of treated mice. These findings may contribute for the discovery of antivenom related valuable pharmaceutical products. The total protein, creatinine, cholesterol and triglyceride levels did not show any significant variations which suggest that the venom has short term-reversible effect in mouse model at sub lethal dose.

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