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# CHANGES IN SERUM COMPONENTS INDUCED BY VENOMS OF MARINE ANIMALS

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## ABSTRACT

Serum enzyme and chemical component levels were investigated in a rut model after eaperimenlal envenomation by venoms of coelentrate, *Physalia* species, four sea-snakes, *Hydrophis spiralis, H. Cyanocinctus, H. Lapemoides and Lapesmis* curtus and a gastropod mollusc *Conus coronatus*. The LD50s through i.v mum were found to be 4.2 mg/kg for Physalia, 0.4 mg/kg for H. spiralis 0.60 mg/kg for H.cyanocinctus, 0.60 mg/kg for *H. lapemoides*, 0.70 mg/kg for *L. eurfus* and 2.9 mg/kg for *C. ceoronaius.* Marked elevation of serum enzyme levels was induced by sea-snake and Physalia venom while *C. ceoronatus* venom showed no significant change in serum levels. The results also indicate gross morphological changes in liver, spleen, gall bladder, lungs and heart by *Physalia* and sea snake venoms.

## INTRODUCTION

It is widely recognised that the venoms of marine animals; sea- snakes, coelentrates, mussel and *Conus* species of phylum mollusca cause a diverse array of clinical manifestation. Among all these venomous species. sea-snakes are most lethal and known to be more toxic than terrestrial snakes (Tu and Hong, 1971).

Generally, envenomation by these animals leads to hematological disorders, paralysis, tissue necrosis etc., besides biochemical, pharmacological and morphological changes in vital organs (Marsden and Reid, 1961; Reid, 1961). Changes in blood components (mainly serum and plasma enzymes) were also observed in cases of mild to severe envenomation by coelentrates and sea- snakes (Muhvich *et al*, 1991; Reid, 1961; Williamson *et* at, 1988). These changes are reported to be associated with cellular necrosis, muscle distraction and organ dystropohy. Each condition is either induced by whole venom or one or several components that comprise the venom.

Several species of sea- snake, coelentrates, bivalves and *Conus* are quite abundant at the coastal areas of Karachi and remain a continuous health hazard for the community. Although no fatality has been reported, several hundred people are effected by these marine organisms every *ye*ar (Alam and Qasim, 1992 a,b).

The purpose of present study is to find out the target organs effected by their venom through analysis of blood components in an animal model. The

venomous species selected for this study belong to the phylum coelentrata (*Physalia* species), sea- snakes (*Hydrophis spbalis, H.* cy*anocincfus, H Lamepoides, Lapemis* aunts) and a gastropod (*Coitus coronolus)* from phylum molluscs.

## MATERIALS AND METHODS

Albino rats of either sex (150-200 gms) were housed in cages and allowed to eat food and *water* ad libitum. All venomous animals were collected in 1991 from coastal areas of Karachi. Venoms were extracted from *Physalia* species by method of Othman and Burnett (1990), from Con= species by the method of Cruz *et al* (1976) and from sea- snake by milking techniques. All venoms were lyophilized and stored at -24°C in ampules. Group of ten rats were injected i.v. with each type of venom sample at different doses and $LD_{50}$ was determined by plotting log dose m percent death. For enzymological studies, venom was dissolved in saline and diluted to reach sub-lethal dose (1/2 and 1/3 of $LD_{50}$). All venom solutions were injected i.v. in a final volume of 1 ml while control rats were injected with 1 ml saline. After 24 hours, animals were sacrificed by ether/chloroform anesthesia and the abdomen was opened immediately. Blood was collected from heart and then the organs (hears kidneys, liver, hears lungs, spleen) were removed for gross morphological studies. Serum was obtained in silicon coated tubes and frozen immediately for enzyme assay and chemical component analysis. The serum enzymes studied were, creatine phosphokinase (CPK). lactate dehydrogenase (LDH), a-glutamyl transpeptidase (GGT), glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), alkaline phosphatase (ALP) and /3-amylase (AMY). The serum chemical components studied were glucose, creatinine, direct and total bilirubin, albumin, blood urea nitrogen, cholesterol, triglyceride, total lipid and total protein. All these parameters were estimated by Hitachi 705 biochemical autoanalyzer using standard kits of enzyme substrate from Boehringer (Germany) according to the manufacturer's instructions. In some cases rats were sacrificed after 48 hours (if not dead) to observe time-dependent changes in blood components and compare it with 24 hours changes.

## RESULTS

The i.v. $LD_{50g}$ of seven venom samples were found to be 4.2mg/kg for *Physalia* species. 0.40 mg/kg for *H.spiralis,* 0.60 mg/kg for H.cyanocinctus. 0.60 mg/kg for *H. lapemoides.* 0.70 mg/kg for *L.cwlus* and 2.9 mg/kg for *Cco*ro*nwus.* However LDs& determined previously of *Physalia* and sea-snake venoms by i.p. route showed a slightly higher dose than i.v. route but induced similar symptoms and death within 12 hr (Alam and Qasim, 1992a,b). The rats receiving 1/2 $LD_{50}$ doses *were* found in critical condition after 20th hr but the group receiving 1/3 $LD_{50}$ survived upto 48 hr.

Changes in serum components after experimental envenoming in rats by venomous marine animals are summarized in Table I and II. Similar changes were observed in animals injected with either 1/2 $LD_{50}$ or 1/3 $LD_{50}$ though the degree of change was dose-related in former while time-related in later. The venom of sea-snake *H. spiralis* was found to be the most potent ($LD_{50}$ 0.40 mg/kg) since it markedly elevated the serum enzyme levels as compared to other venom samples. The concentration of serum chemical components; glucose, total and d[i]rect bilirubin, blood urea nitrogen was also markedly elevated while that of total protein, albumin, total lipid, cholesterol and triglyceride were declined after i.v. injection of *H. spiralis* venom as compared to controls. The venom of sea- snakes *H. cyanocinclus, H. lapemoides, L. curtus* and *Physalia*, on the other hand, showed a somewhat similar changes in serum enzymes and chemical component levels.

Marked elevation and decline in serum components in all cases except *Conus* indicated that the venom mainly targeted heart, liver, kidneys, blood vessels and less commonly lungs, digestive tract and spleen etc. The least changes were observed with *Conus* venom which showed no significant change. The animals receiving sea-snake venom showed liver with their surface becoming rough and colour change from reddish brown to patchy brownish black. Spleen was dark brown while red or brown patches were observed on heart in few cases. Swollen gall bladder and a jaundiced liver was more frequent in case of *Physalia* venom; the colour of lungs changes from light pinkish-yellow to yellowish white with marked reddend veins.

## DISCUSSION

Ouantitation of serum and plasma enzymes and other chemical components have long been known to be significant for general diagnosis of cellular necrosis, muscle distruction and organ dystrophy; in general of a disease or disease like condition. For example CPK present in skeletal and heart muscle and elevated in case of myocardial infarction, muscle dystrophy and cerebral infarction (Rodwell, 1990). Similarly SGOT, SGPT, GGT, LDH, ALP, AMY (liver, brain and muscle enzymes) level elevated in case of myocardial infarction, cirrhosis of liver, acute and chronic hepatitis, glomerulonephritis, acute pancreatitis, muscular dystrophy, dermatomyositis and general tissue necrosis including that of other organs as well (Rodwell, 1990).

The levels of normal chemical components of serum or plasma such as total lipid, cholesterol, triglyceride, total protein, albumin, creatinine, glucose, blood urea nitrogen, total and direct bilirubin are elevated or decreased in acute hepatitis or hepatic insufficiency, nephritis, congestive heart failure, malabsorption syndrome and adrenal insufficiency (Rodwell, 1990).

Table 1

**Changes in serum enzyme levels in rats after experimental envenomation by venomous marine animals.**

| | $V_{PP}$ | $V_{HS}$ | $V_{HC}$ | $V_{HL}$ | $V_{LC}$ | $V_{CC}$ | C |
|---|---|---|---|---|---|---|---|
| CK | 77.10 ±1.72 | 84.90 ±1.90 | 72.80 ±2.60 | 69.20 ±2.04 | 77.31 ±1.65 | 37.21 ±2.40 | 30.24 ±2.14 |
| LDH | 2604.5 ±119.2 | 1995.8 ±58.70 | 2005.8 ±53.21 | 2407.8 ±94.45 | 1656.6 ±74.79 | 178.0 ±5.77 | 174.5 ±6.00 |
| SGOT | 244.0 ±20.60 | 836.80 ±21.36 | 693.50 ±33.75 | 728.70 ±25.80 | 266.17 ±13.02 | 53.15 ±2.64 | 43.90 ±3.00 |
| SGPT | 65.15 ±5.65 | 180.8 ±3.50 | 173.4 ±7.64 | 168.4 ±4.44 | 152.7 ±6.54 | 43.14 ±3.00 | 16.35 ±1.78 |
| GGT | 158.35 ±4.04 | 291.3 ±11.00 | 156.3 ±5.35 | 145.90 ±5.20 | 153.90 ±6.20 | 42.04 ±3.80 | 28.70 ±2.62 |
| ALP | 163.8 ±6.35 | 267.50 ±12.93 | 137.3 ±5.00 | 134.70 ±9.63 | 130.0 ±6.00 | 60.60 ±5.41 | 59.10 ±2.86 |
| AMY | 1404.6 ±68.61 | 2220.2 ±84.14 | 1908.0 ±46.57 | 172.0 ±12.27 | 1506.0 ±87.75 | 139.40 ±7.25 | 104.6 ±3.60 |

Values are expressed as mean ± S.E.M. Control n = 30

**Enzyme values are expressed as follows;**

CK (creatine phosphokinase), LDH (lactate dehydrogenase), SGOT (serum glutamate-oxaloacetate transminase), SGPT (serum glutamate-pyruvate transaminase), GGT (δ-glutamyl transpeptidase), AMY (amylase), ALP (alkaline phosphatase) = IU/L.

$V_{PP}$ = *Physalia* venom, $V_{HS}$ = *H. spiralis* venom, $V_{HC}$ = *H. cyanocinctus* venom $V_{HL}$ = *H. lapemoides* venom, $V_{LC}$ = *Lapemis curtus* venom, $V_{CC}$ = *Conus coronatus* Venom, C = control.

Table 2
Changes in serum chemical component levels in rats after experimental envenomation by venomous marine animals.

| | $V_{PP}$ | $V_{HS}$ | $V_{HC}$ | $V_{HL}$ | $V_{LC}$ | $V_{CC}$ | C |
|---|---|---|---|---|---|---|---|
| GLU | 146.0 ±5.70 | 248.5 ±21.45 | 167.3 ±5.90 | 164.4 ±8.03 | 128.6 ±7.00 | 77.75 ±5.50 | 90.10 ±3.50 |
| CREAT | 3.50 ±0.75 | 2.95 ±0.43 | 1.52 ±0.12 | 1.25 ±0.04 | 1.47 ±0.13 | 0.69 ±0.06 | 0.43 ±0.09 |
| T-BIL | 4.36 ±0.52 | 6.80 ±0.53 | 4.60 ±0.65 | 4.87 ±0.58 | 4.00 ±0.50 | 1.17 ±0.01 | 0.63 ±0.04 |
| D-BIL | 4.72 ±0.05 | 6.84 ±0.57 | 5.84 ±0.50 | 3.92 ±0.56 | 4.58 ±0.71 | 1.55 ±0.08 | 0.78 ±0.09 |
| BUN | 100.4 ±6.80 | 196.0 ±11.16 | 146.8 ±7.30 | 120.50 ±9.40 | 124.6 ±5.60 | 14.50 ±1.80 | 15.50 ±1.60 |
| T-PRO | 3.00 ±0.48 | 2.90 ±0.33 | 3.72 ±0.42 | 4.00 ±0.23 | 3.90 ±0.43 | 4.40 ±0.40 | 7.40 ±0.24 |
| ALB | 1.42 ±0.18 | 1.19 ±0.12 | 1.50 ±0.11 | 1.55 ±0.14 | 2.05 ±0.12 | 2.80 ±0.23 | 2.90 ±0.32 |
| TLIP | 240.2 ±22.40 | 148.0 ±7.40 | 253.5 ±18.20 | 219.0 ±10.22 | 229.0 ±16.60 | 590.4 ±18.0 | 650.5 ±15.74 |
| CHOL | 104.10 ±8.00 | 95.0 ±5.50 | 105.5 ±5.40 | 94.00 ±5.50 | 103.0 ±6.04 | 167.0 ±6.10 | 197.0 ±5.30 |
| TAG | 50.90 ±7.30 | 15.30 ±2.12 | 29.30 ±4.30 | 67.00 ±4.70 | 23.90 ±2.80 | 117.9 ±4.30 | 100.2 ±7.10 |

Values are expressed as mean ± S.E.M. Control n = 30
**Serum biochemical constituent level are expressed as follows;**
GLU (glucose), CREAT (creatinine), T-BIL (total bilirubin), D-BIL (direct bilirubin), BUN (blood urea nitrogen), CHOL (cholesterol), TAG (triglyceride), T-LIP (total lipid) = mg/dl; whereas T-PRO (total protein) and ALB (albumin) = g/dl. $V_{PP}$ = *Physalia* venom, $V_{HS}$ = H. spiralis venom. $V_{HC}$ = *H. Cyanocinctus* venom. $V_{HL}$ = *H. lapemoides* venom, $V_{LC}$ = *Lapemis curtus* venom, $V_{CC}$ = *Conus coronatus*. Venom, Control.

Serum enzymes examined in our case in a rat model showed elevated levels of CPK, LDH, SGOT,SGPT, GGT and ALP dearly indicating the presence of necrosis or cellular damage in liver, heart, kidneys and lungs induced by venom from *Physalia* and sea-snakes. Elevated levels of serum enzyme confirmed previous findings about severe morphological alterations in heart, kidneys, liver, lungs and skin induced by *Physalia* and sea- snake venom (Alam and Qasim, 1992a,b). Coma venom did not exhibit marked elevation of serum enzymes since Coma venom and its components are primarily neurotoxic and cause death by neurological collapse rather than tissue or organ damage.

The venoms of coelentrate and sea-snake have been reported to be potent nephrotoxic, hepatotoxic and cardiotoxic agents which not only severely damage these organs, but if untreated, cause paralysis and death (Marsden and Reid, 1961; Muhvich *et al*., 1991; Reid, 1961; Williamson *et al*, 1988). Elevated levels of SGOT, SGPT, CPK and LDH was also reported for victims of Physalia physalis envenoming (Williamson et al., 1988) while that of SCOT and SGPT after experimental envenomation in rats by Chrysaom quinquecinha venom (Muhvich *et al*, 1991). Marked elevation of serum GOT, CPT, LDH, add phosphatase and ALP as well as hepatic ALP was reported in mice receiving lethal factor isolated from a starfish *Acanthaster planci* (Shiomi *et al*, 1990). Gross morphological changes in liver and gall bladder and histopathological changes in liver were also observed in mice by *A. planci* lethal factor (Shiomi *et al.*, 1990).

Decreased levels of total lipid, triglyceride, cholesterol, total protein, albumin and elevated levels of glucose, creatinine, total and d[i]rect bilirubin and blood urea nitrogen indicates the possibility of hepatitis, glomerulonephritis (and renal failure), malabsportion syndrome and myocardial necrosis. Reid (1961) also reported raised blood creatine and blood urea levels in victims of sea-snake bite with severe renal damage. Increased hemoglobin and blood urea nitrogen level was also reported for victims of Physalia physalis envenomation (Williamson *et al*., 1988). Hyperglycemia and elevated amylase and plasma immunorcactive cation trypsin levels were also reported for victims of scorpion *Leiuna quinquesaiatus* (yellow scorpion), *Tityus tinitatis*, and *T. senulatus* stings indicating acute pnacreatitis (Sofer *et al*., 1991).

In conclusion the estimation of enzymes and biochemical components from serum of experimentally envenomated rats described in this communication gives a clear picture of organs effected by marine venoms in general and confirmed the[i]r necrotic potency against vital organs in particular.

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