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Pneumotoxic Activity of Crude Venom and A Cytolytic Protein, PuTX-IVC, from a Coelenterate, *Physalia utriculus* (Blue Bottle)

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Abstract.- Envenomations by jellyfishes are a menace for beach-goers, fisherman and swimmers during summer months in coastal areas of the world, including Karachi, Pakistan. Accidental stings and envenomations in human results in spasms, utricaria, dermonecrosis and in extreme cases, cardiac and respiratory distress. The present communication describes the pneumotoxic activity of *Physalia utriculus* crude venom and a purified cytolytic protein, PuTx-IVC. Both crude venom and cytolysin induces mild to marked alteration of biochemical characteristics of pleural fluid, evident by elevation of total protein, albumin, globulin, and lactate dehydrogenase contents and broncho-dialatation and infiltration of inflammatory cells in alveoli of lungs. Results confirmed the onset of one of the most prominent clinical features of human jellyfish envenomations, which is respiratory distress. However, it is suggested that pneumotoxic activity of *P. utriculus* crude venom and the purified component, PuTx-IVC, may not be a direct one but a consequent manifestation created by internal hemolysis and hemorrhage of blood vessels, causing damage and blocking of heart ventricles and valves. This subsequently results in accumulation of fluid in pulmonary cavities, causing dyspnea (respiratory distress) and edema.

Key Words: Jellyfish, crude venom, cytolysin, pneumotoxic activity.

INTRODUCTION

Envenomations by coelenterates, especially jellyfishes, are a menace for beach-goers, fisherman and swimmers during summer months of May to September (Alam and Qasim, 1991; Alam *et al.,* 1992). Accidental stings and envenomations in human results in spasms, utricaria, dermonecrosis and in extreme cases, cardiac and respiratory distress, and in few cases, fatality (Alam and Qasim, 1991, 1996; Burnett and Gable, 1989; Stein *et al.,* 1989; Exton *et al.,* 1989; Williamson, 1990; Williamson *et al.,* 1996). Such clinicopathological conditions are attributed to the presence of toxic components in coelentrate venom (Alam *et al.,* 2002; Burnett and Calton, 1977; Chung *et al.,* 2001; Gondran *et al.,* 2002; Marques *et al.,* 2002). Most of the coelentrates, and specially those known as jellyfish, has the distinction from other venomous animals to possess toxins with a wide range of molecular weight and diverse array of clinical, patho-physiological and pharmacological activities (Alam and Qasim, 1996; Alam *et al.,* 2002; Burnett and Calton, 1977; Edwards and Hessinger, 2000; Gondran *et al.,* 2002; Mariottini *et al.,* 2002; Monastyrnaya *et al.,* 2002; Menendez *et al.,* 1990; Russell, 1984; Sun *et al.,* 2002).

Physalia species (Portuguese man-of-war or blue bottles), both multitentacular, *Physalia physalis* and uni-tentacular *Physalia utriculus,* have distribution in the tropical and sub-tropical region, including Karachi, Pakistan (Alam and Qasim, 1991-1993; 1996; Alam *et al.,* 2002; Williamson *et al.,* 1996), and is a common health hazard, every year, for beach-goers and fisherman. In summer of 2003 alone, 250 persons per day were inflicted with *Physalia* stings on Clifton and Sandspit beaches causing mild to severe utricaria, popular eruptions, itching, stomach ache, spasms, respiratory distress and sweating. The studies carried out on *Physalia* venom and nematocyst extracts during last three decades, extensively focused on the presence and confirmation of clinico-pathological and general biological activities and the components,

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responsible for it (Alam and Qasim, 1996; Burnett *et al.,* 1988; Burnett and Calton, 1974a,b, 1976; 1977; 1987; Calton *et al.,* 1973; Edwards Hessinger, 2000; Lin and Hessinger, 1979; Menendez *et al.,* 1990; Tamkun and Hessinger, 1981).

We have also reported the biochemical and biological properties of crude venom from *Physalia physalis* and *Physalia utriculus* (Alam and Qasim, 1991-1993; Alam *et al.,* 1992; 1993) and purified cytolytic proteins from *Physalia utriculus* (Alam and Qasim, 1996; Alam *et al.,* 2002). The present communication describes the pneumotoxic activity of crude venom and a cytoloytic protein PuTx-IVC from *P. utriculus* in albino rats. The purified protein was noted to be more potent pneumotoxic as compared to crude venom, suggesting that the respiratory distress, which often occurs in cases of severe *Physalia* stings, may be caused by the presence of such pneumotoxic components in jellyfish venom, either directly or indirectly.

MATERIALS AND METHODS

Materials

The jellyfish species, *Physalia utriculus* (Pu), were collected during summer months of May to August. Tentacles were cut from live specimen and refrigerated at 4°C for 24 hours for autolysis. Venom extraction was carried out by established techniques (Endean *et al.,* 1969; Long and Burnett, 1989) whereas PuTx-IVC, purified by the method of Alam *et al.,* (2002) was used as Cytolysin. A total of 72 albino rats (average wt 150 gm; either sex) were used in the present study. Eight animals each, were used as controls for two test groups crude venom (CV) and PuTx-IVC. The remaining 56 animals were equally divided for both test groups and housed four per cage and allowed to consume water and food *ad libitum.*

Methods

One LD_{50} doses, 4.60±0.10 mg/kg and 1.19±0.03 mg/kg, respectively, of crude extracted venom and purified cytolytic protein PuTx-IVC were diluted upto 1/10 with 0.85% NaCl to extend lethal time, and administered intraperitoneally (*i.p.*) in albino rats. For biochemical and histopathological studies, four groups of animals were sacrificed at specified time intervals (12, 24, 36, 48, 60, 72, 84 hours), their abdomen were cut open and pleural fluid (PF) was aspirated from both lungs cavity by micro-syringe connected to special heparinized vacuum tubes. Care was taken to avoid contamination with cellular/tissue debris and blood. After aspiration, lungs were removed and fixed in 10% formalin for histopathological examination. Control animals were given 1.5 ml of 0.85% NaCl *i.p*. and sacrificed after 48 hrs. Aspirated PF was centrifuged (and diluted where needed) and clean suspension was used for biochemical studies. Biochemical characterization was carried out on automated Hitachi Chemistry Analyzer 912 (Roche Diagnostics, Basel) with double calibration and both normal and pathological controls. Total protein, albumin, globulins (all in gm/dl), glucose (mg/dl) and lactate dehydrogenase (LDH, IU/L) were estimated in triplicate and results are expressed as mean±S.E. Histopathological examinations were carried out by the methods described earlier (Alam and Qasim, 1996; 2002; Alam *et al.,* 1993). The results were statistically analyzed using Student's t-test with significant P value of less than 0.01.

RESULTS

The results are summarized in Figures 1-5. Within three to five hours after administration of sub-lethal doses of either crude venom or PuTx-IVC, the animals begins to tremble, with slight to moderate tremor, and sometimes crawling movements. After eight to twelve hours, mild convulsions starts in some rats that, lasts for four to six hours in groups received "CV" and 12 hours in groups received PuTx-IVC. Laborious breathing, respiratory distress, increased heart rate and redness of eyes and skins (in some animals) also found setting within six to eight hours in “CV” groups and two to three hours in purified cytolysin group. Mild form of respiratory distress and tremor and moderate to slightly marked form of the same clinical manifestations remains upto 48 hours in “CV” and PuTx-IYC groups, respectively. All clinical signs subsided, except withdrawal, after 60 hours from all test groups of animals. None of the rats in test groups died during the course of the present study. However, weight loss, lose motion

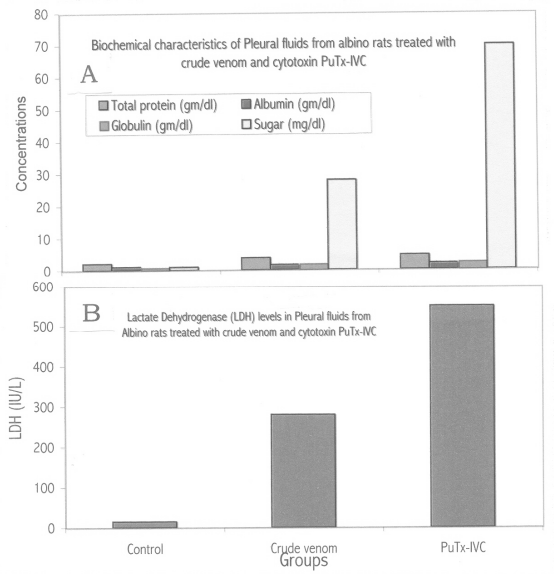

Fig. 1. Biochemical characteristics of pleural fluids from albino rats treated with crude *Physalia* venom and purified cytolysin PuTx-IVC. The results are expressed as Mean±S.E. A, changes in total protein, albumin, globulin and sugar contents as compared to controls; B, changes in LDH levels.

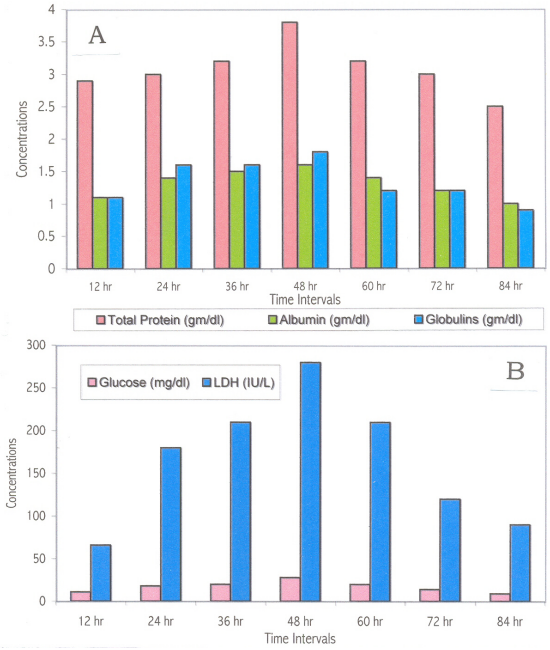

Fig. 2. Time-dependent changes in biochemical characteristics of pleural fluids from albino rats treated with *Physalia* venom. The results are expressed as Mean±S.E. A, comparable changes in total protein, albumin and globulin contents as compared to controls; B, Changes in LDH and glucose levels.

and traces of blood in feces and urine of some animals were evident. On gross examination, lungs seem pale-reddish to yellowish-brown and puffy, mostly in groups treated with purified cytolysin. Only few in "CV" groups showed any gross anatomical alterations in lungs. Pleural fluids, aspirated from lungs with certain difficulty, were pale to dark yellowish in color, in treated groups of animals as compared to off-white and translucent in control groups.

Biochemical analysis of pleural fluid (Fig. 1A,B) revealed marked alteration of characteristics chemistry in both "CV" (Fig. 2A,B) and purified cytolysin PuTx-IVC groups (Fig. 3A,B), however, significantly more in later group than the former. Gradual marked elevation of total proteins, albumin, globulins, glucose and LDH levels were noted at 48 hours in both groups (Figs. 1-3), which subsided gradually in proceedings 48 hours. However, the levels remains abnormally elevated as compared to control groups. Histopathological examinations of lungs showed marked infiltration of inflammatory cells within alveoli (Fig. 4B; Fig. 4A = control). In groups treated with purified cytolysin PuTx-IVC (Fig. 5A,B), also showed disfigurement and disorientation of bronchus with disquamation of epithelia. Marked infiltration of inflammatory cells was also noted at epi-bronchus in most of the animals treated with purified PuTx-IVC (Fig. 5B).

DISCUSSION

Coelenterate envenomations, either by jellyfish or sea-anemone, are related to several clinical signs and symptoms with mild to marked manifestations of utricaria, papular eruptions, spasms in extremities, tremor, itching, heavy perspiration and respiratory distress (dyspnea) (Alam and Qasim, 1991, 1996; Alam *et al.,* 1992, 1993, 2002; Burnett and Calton, 1977; Burnett and Gable, 1989; Marques *et al.,* 2002; Stein *et al.,* 1989; Williamson 1990; Williamson *et al.,* 1996). Jellyfish envenomation is a continuous health

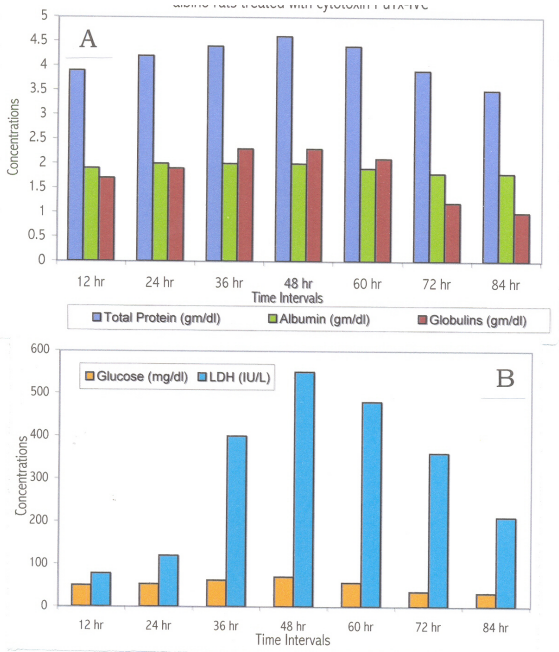

Fig. 3. Time-dependent changes in biochemical characteristics of pleural fluids from albino rats treated with purified cytolysin PuTx-IVC. The results are expressed as Mean±S.E. A, comparable changes in total protein, albumin and globulin contents as compared to controls; B, changes in LDH and glucose levels.

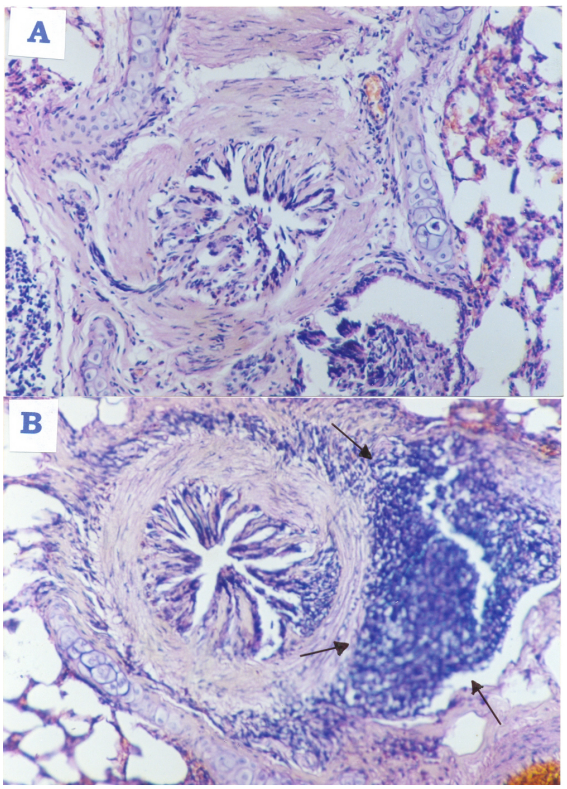

Fig 4: Histopathological changes in lungs of albino rats treated with *Physalia* venom and purified cytolysin PuTx-IVC. A, control with normal morphology of bronchus and alveoli [H&E, x 150], B, marked infiltration of inflammatory cells in epi-bronchus (arrows) [H&E, x 750].

hazard for sea-side cities, especially in summer months. In Karachi also, this menace cause inconvenience to a large number of beach-goers and fisherman (Alam and Qasim, 1991, 1992; Alam *et al.,* 2002). In last year alone, an average of 250 persons/day were inflicted with *Physalia* stings resulting in severe pain, itching utricaria, redness, respiratory distress, numbness in extremities, mostly in children and vomiting in some cases. Studies carried out by our group on *Physalia* venom for more a decade since 1991, revealed that the venom, although, not as potent to inflict any fatal conditions, but still have the ability to induce mild to marked clinical conditions in experimental animals which seemingly manifested in humans after accidental envenomation (Alam and Qasim, 1991, 1996; Alam *et al.,* 1992, 1993, 2002). Venom of *Physalia utriculus,* which is the mostly prevails species of *Physalia* on Karachi beach, have several high molecular weight, heat-labile, cytolytic protein components which induces lethal, hemolytic, hemorrhagic, edema-inducing and cytotoxic manifestations in experimental animals.

Present study describes the pneumotoxic activity of crude extracted venom from *P. utriculus* and its cytolysin PuTx-IVC in albino rats. The results confirmed the onset of one of the most marked clinical signs appears in accidental human envenomation, which is dyspnea (respiratory distress or breathlessness). However, it seems that this pneumotoxic activity is not a direct one but related to the hemolytic activity or cardiac-renal insufficiency constituted by sub-lethal doses of both crude venom and cytolysin PuTx-IVC. The etiology and patho-physiological explanation is such that hemolysis and internal blood vessel hemorrhage caused by experimental envenomation may result in

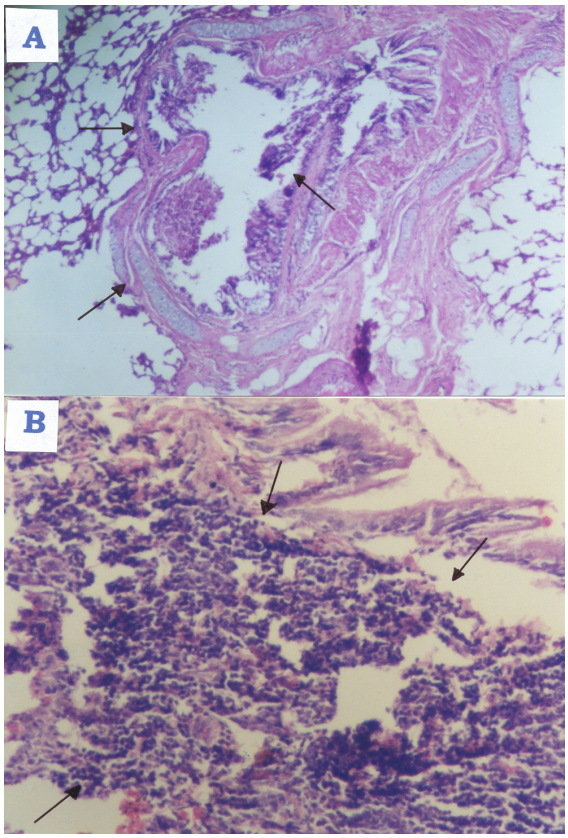

Fig 5: Histopathological changes in lungs of albino rats treated with *Physalia* venom and purified cytolysin PuTx-IVC. A, marked dilatation of bronchus with epithelial disquamation [H&E, x 350], B, marked infiltration of inflammatory cells in epi-bronchus (arrows) [H&E, x 750].

cardiac abnormality or valve damage. To cope with internal hemolysis and hemorrhage, the automatic coagulation mechanism comes in action, which may either cause blood stasis or possible thrombus formation. The fibrillating heart valves cause an increased risk of embolic stroke. This clinical condition manifested most prominently in lungs, although the function of the kidneys and brain may also be markedly impaired (Cotran *et al.,* 1994; Schoen, 1994). The last two pathological conditions were also confirmed in our study by convulsive animals and those, which urinated with traces of blood, and internal bleeding. With the progressive damming of the blood within the pulmonary circulation, pressure in pulmonary veins increases. This results in pulmonary congestions and edema, manifested as heavy and wet lungs. A slightly larger lungs and sticky, as compared to controls, were noted in our study in the groups treated with CV and PuTx-IVC. These anatomical changes strikingly manifested clinically as Dyspnea or respiratory distress (breathlessness), observed both in accidental human envenomation by jellyfishes and experimental animals. Such cardio-pulmonary effects also cause renal insufficiency, causing renal perfusion, activating Renin-angiotensin aldosterone system. This induces retention of salt and water, resulting in consequent expansion of the interstitial fluid and blood vessels. This compensatory reaction contributes again to the pulmonary edema (Schoen, 1994).

In conclusion, we have reported pneumotoxic activity of *Physalia utriculus* crude venom and a purified cytolytic protein, PuTx-IVC. Both crude venom and cytolysin induces mild to marked alteration of biochemical characteristics of pleural fluid and histology of lungs. Results suggest that pneumotoxic activity of venom and its component may not be a direct one but a consequent manifestation created by internal hemolysis, hemorrhage of blood vessels causing damage and blocking of heart ventricles and valves. This results in accumulation of fluid in pulmonary cavities, causing dyspnea and edema. The studies are in progress to evaluate renal effects as well as neurological activities of *Physalia* venom and its toxic components.

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