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Nephrotoxicity Induced in Experimental Animals by Single Dose of A Shellfish Cytotoxin $PvHTxII_E$

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Abstract.- We have described the nephrotoxic effect of a contaminated shellfish cyto-hepatotoxin, $PvHTxII_E$, after a single dose in experimental animals. $PvHTxII_E$ toxin [LD_{50} of 0.40 ml/kg (48 µg/150 g, b.w.)] was injected intraperitoneally (i.p.) in experimental animals and clinical signs were noted periodically at 6, 12, 24, 36 and 48 hrs. Blood sugar, urea, creatinine, chloride, sodium, potassium, bicarbonate, protein, osmolality, enzymes (LDH, CK, AST, ALT, ALP), liver function tests (γGT, AST), circulating calcium level and its compensatory ion phosphate, urinary urea, creatinine, sodium, protein and osmolality were analyzed at different time intervals (6, 12, 24, 36 and 48 hrs) to assess the onset and extent of renal injury. The toxin induced alteration of renal system, manifested through variation in kidney function parameters such as urinary osmolality, urea, creatinine and blood potassium levels. Mild to moderate histopathological changes were also noted in kidney tubules such as epithelial disquamation and dilatation. It was also noted that impairment of renal function, as indicated by abnormal levels of chemical analytes and histopathological changes in kidney tubules, was mainly significant ($P< 0.05$) at 24^{th} hour. It is, therefore, concluded that the nephrotoxic effect induced by $PvHTxII_E$ is not a direct one, but a pathophysiological manifestation occurred during metabolism and elimination of toxin through renal system.

Key Words: Marine toxins, nephrotoxic, hepatotoxin, renal system.

INTRODUCTION

One of the most important families of marine toxins is cytotoxin, predominant in a number of marine fauna (Burnett and Calton, 1977; Russell, 1984; Terao *et al.,* 1988, 1990, 1994; Alam *et al.,* 1992, 1993, 1995a-c; Alam and Qasim, 1997; Rossini *et al.,* 2001). Among these cytotoxins, shellfish toxins are considered as a significant toxicological threat because of its effects on seafood production and consumers health (Sato *et al.,* 2000; Ammons *et al.,* 2001; Freitas de Magalhaes *et al.,* 2001; Nobre *et al.,* 2001; Norris *et al.,* 2002; Vale and Sampayo, 2001). Shellfish toxins include two major classes, diarrhetic shellfish toxin (DSP) and paralytic shellfish toxin (PSP) (Shimizu, 1978; Russell, 1984), produced by toxic phytoplankton *e.g.* dinoflagellates, cyanobacteria and diatoms, that are ingested by shellfish as their food (Ammons and Sampayo, 2001; Chen and Chou, 2001; Park *et al.,* 2001; Vale and Sampayo, 2001). In addition to both classes, several related toxins such as hepatotoxin, enterotoxin and amnesic poison, that cause considerable pathological manifestations, have also been reported (Silkker *et al.,* 1998; Flanagan *et al.,* 2001; Ito *et al.,* 2002; Mehto *et al.,* 2001; Chong *et al.,* 2002; Norris *et al.,* 2002). The etiology and mechanism of action of these toxins have been investigated and reported extensively. Increase in cellular permeability to Ca^{++} ions, alteration in Na/CI channels, morphological changes and cellular disintegration, impairment of enzyme mechanism and bile acid transport system have been demonstrated to be the pathogenesis of shellfish toxins (Terao *et al.,* 1990; Harada *et al.,* 1994; Rossini *et al.,* 2001; Chong *et al.,* 2002). Furthermore, renal insufficiency was also reported as one of the manifestations of shellfish toxins (Hooser *et al.,* 1989; Radbergh *et al.,* 1991; Kotak *et al.,* 1996; Nobre *et al.,* 1999,2001; Ito *et al.,* 2002).

The present communication describes the nephrotoxicity of $PvHTxII_E$, a hepatotoxin isolated from contaminated mussel, *Perna viridis* (Alam and Qasim, 1997), in albino rats after a single dose. The toxin was reported to induce marked hepatotoxicity

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in rats by increasing permeability of cellular calcium and an increase in circulating calcium concentration.

MATERIALS AND METHODS

Lethality and histopathological examination

$PvHTxII_E$ toxin, having an LD_{50} of 0.40 ml/kg (48 µg/150 g, b.w.) in rats, was diluted with deionized water such that the lethality period was extended upto 48 hours. This was done to achieve the proper involvement of kidneys for excretion and elimination. Five groups, each of four male rats were injected intraperitoneally *(i.p.)* with $PvHTxII_E$ and clinical signs were noted periodically at 6, 12, 24, 36 and 48 hrs. Rats from each group were sacrificed at the specified period, blood was collected and processed as described earlier (Alam and Qasim, 1997). Abdominal cavity was cut open and organs were removed, fixed, embedded in wax and processed for histological sections as reported earlier (Alam *et al.,* 1992, 1993). Control group of eight rats was administered *i.p.* injection of normal saline and processed similarly as test group.

Biochemjcal analysis

Blood analytes; sugar, urea, creatinine, chloride, sodium, potassium, bicarbonate, protein; and enzymes (lactate dehydrogenase, LDH; creatine kinase CK; aspartate aminotransferase, AST; alanine aminotransferase, ALT, alkaline phosphatase, ALP) were analyzed by automated chemistry analyzer 912 (Hitachi 912, Roche Diagnostics, Basel) with full calibration and controls of normal and pathological indices. Blood osmolality was also determined and reported as milliosmol/kg as compared to control rats (190±4.8 mosmol/kg). Each test was repeated atleast twice and results were reported as Mean±SE. The blood hepatic parameters (liver function tests profile, gamma-glutamyl transpeptidase γGT and AST) were also analyzed as routine to ensure the existence of hepatotoxicity of $PvHTxII_E$. Circulating calcium level and its compensatory ion phosphate was determined by automated analyzer (Hiatchi 912, Roche Diagnostic, Basel) using standard procedures recommended by the manufacturer, to assess the extent of renal injury.

Urinalysis

To collect the required analytical quantity of urine from rats, each rat, before sacrifice at the specified time period was picked and squeezed from abdomen with slight pressure, which assures their urination. For collection, a small-sterilized glass tube was placed at the penile end, and all urination was carried out in a single tube. The content of tube was centrifuged at 8,000 rpm, separated in three aliquots and analyzed for urinary urea, creatinine, sodium and protein. If quantity collected was more than 2.0 ml, the sample was processed for urinary osmolality and reported as milliosmol/kg in comparison with control group (100±5.68 mosmol/kg).

Statistical analysis

The results were statistically analyzed using Microsoft computer program, SSP version 10 (Microsoft Inc, USA) by applying Student's t-test, one and two way ANOVA and repeated ANOVA with significant *P* value less than 0.05 ($P < 0.05$).

Chemicals and animals

All chemicals for analysis are reagent grade and obtained from commercial source. Twenty-eight male albino rats, apparently healthy (150-200 gm) were housed as 4/cage, and allowed to consume water and feed *ad libitum.*

RESULTS

Assessment of kidney and hepatic functions

Figures 1-3 describe the time-dependent alteration in hepatic and kidney function in test groups of animals. Several blood and urinary parameters were determined to evaluate the role of kidneys and the concomitant effect of $PvHTxII_E$ on kidney function. Initially, all parameters; osmolality, urea, creatinine, chloride, sodium, potassium, bicarbonate, and protein were found at a comparative concentration to controls. However, disruption of renal function starts at 12th hour which remains impaired, although non-significantly, upto 48 hours. The parameters most significantly altered ($P < 0.01$) were osmolality, urea, creatinine, potassium and calcium (Fig. 1A, B). Marked impairment of renal function, as indicated by

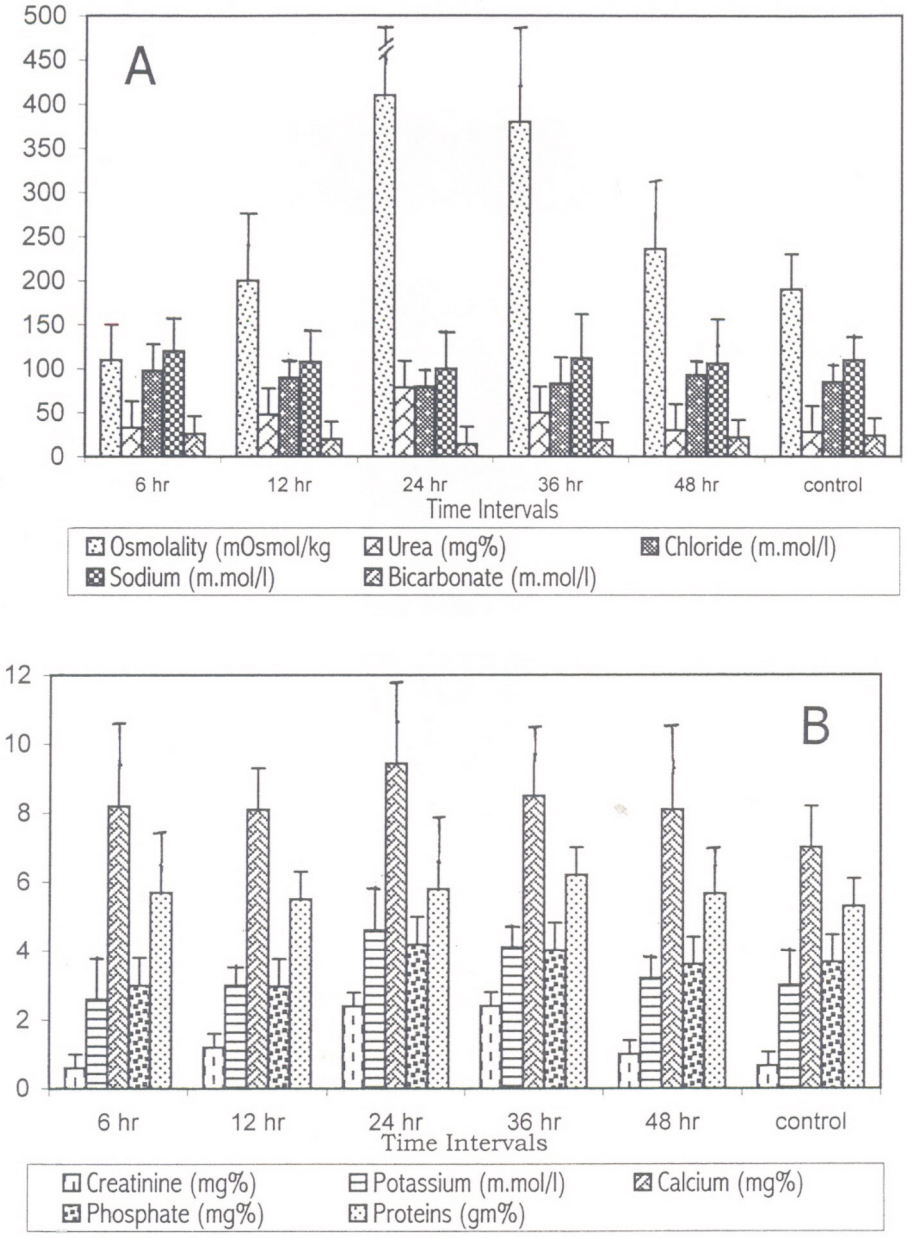

Fig. 1. Time-dependent changes in kidney function test profile in blood of albino rats after a single dose of cyto-hepatotoxin $PvHTXII_E$. The results are expressed as Mean±S.E. (A): Osmolality, Urea, Chloride, Sodium, Bicarbonate. Osmolality compared with control, significant alteration ($P < 0.05$) at 6 hr, 12 hr, 48 hr; $P< 0.01$ 24 hr, 36 hr; Urea $P < 0.05$ at 12 hr to 36 hr; Chloride, Sodium and Bicarbonate, NS. Comparison within different time intervals; Osmolality 6 hr vs 12 hr and 24 hr, and 36 hr vs 48 hr $P < 0.05$; 6 hr, 12 hr vs 24 hr and 36 hr $P < 0.001$. Urea, Chloride, Sodium and Bicarbonate, Non-significant (NS) (B): Creatinine, Potassium, Calcium, Phosphate, and protein. Comparison with control; creatinine 12 hr to 48 hr significance alteration at $P < 0.05$; Potassium 24 hr and 36 hr $P < 0.01$; Calcium 24 hr $P < 0.05$; Phosphate 6 hr and 12 hr $P < 0.05$; Proteins NS. Comparison within different time intervals in test group, NS.

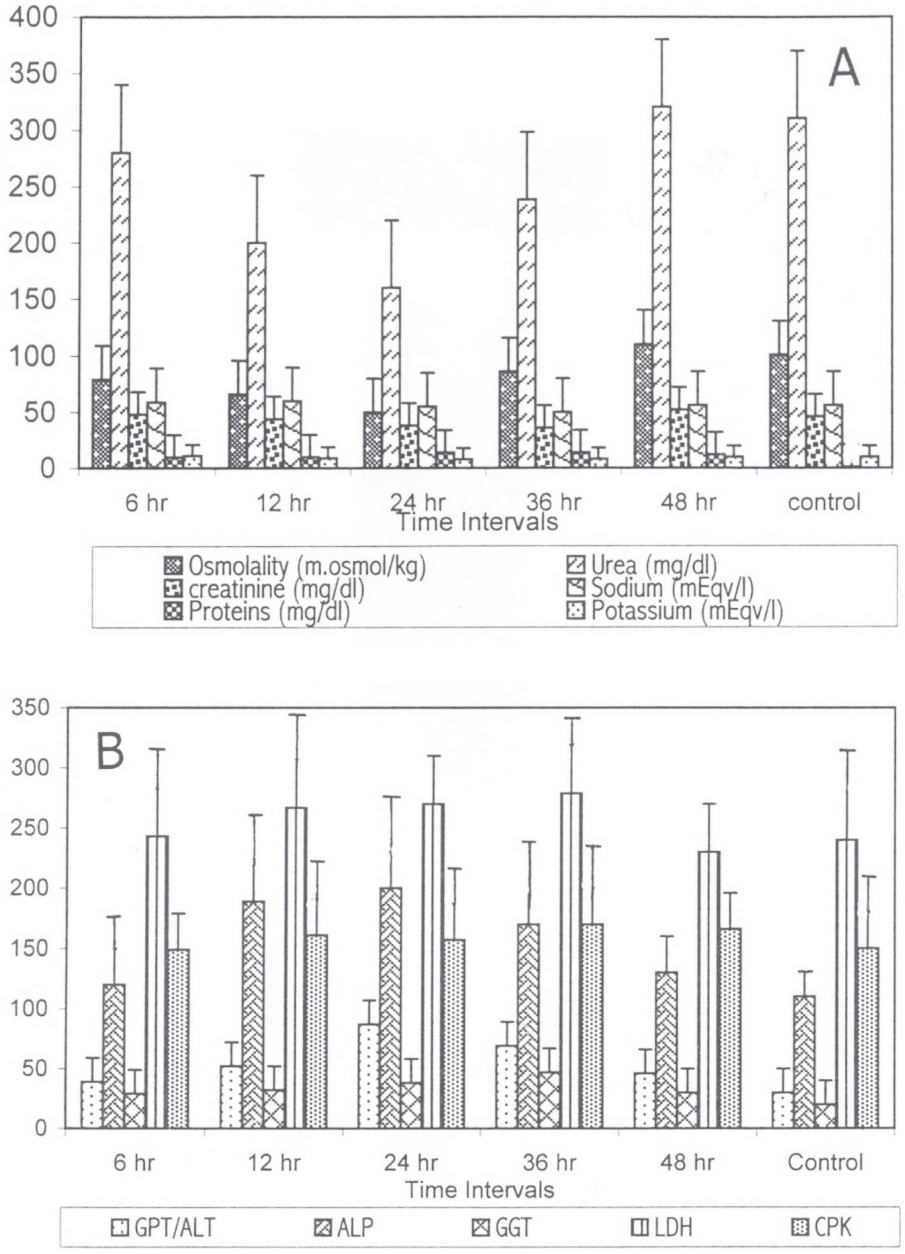

Fig. 2. Time-dependent changes in kidney function test profile in urine (A) and hepatic (B) parameters (IU/L) of albino rats after a single dose of cyto-hepatotoxin $PvHTXII_E$. The results are expressed as Mean±S.E. (A): Urinary Osmolality, Urea, Creatinine, Sodium and Potassium. Comparison with control; Osmolality 6 hr ($p< 0.05$), 12 hr to 36 hr ($P < 0.01$); Urea 12 hr to 36 hr ($P < 0.05$); Creatinine, Potassium, sodium, non-significant (NS). Comparison within different time intervals of test group, Osmolality and Urea 24 hr vs 6 hr and 12 hr, 36 hr and 48 hr $P <0.05$. Creatinine, Sodium and Potassium-NS. (B): Hepatic (ALT, ALP, GGT, LDH), and Muscle (CPK) Parameters. Comparison with control; ALT 24 hr and 36 hr $P < 0.05$, ALP 12 hr to 36 hr $P < 0.05$, GGT and LDH 36 hr $P < 0.05$. CPK-NS. Comparison within different time intervals in test group; ALT 6 hr, 12 hr, 48 hr vs 24 hr $P < 0.05$; ALP 6 hr vs 24 hr $P < 0.05$; GGT, LDH, CPK-NS.

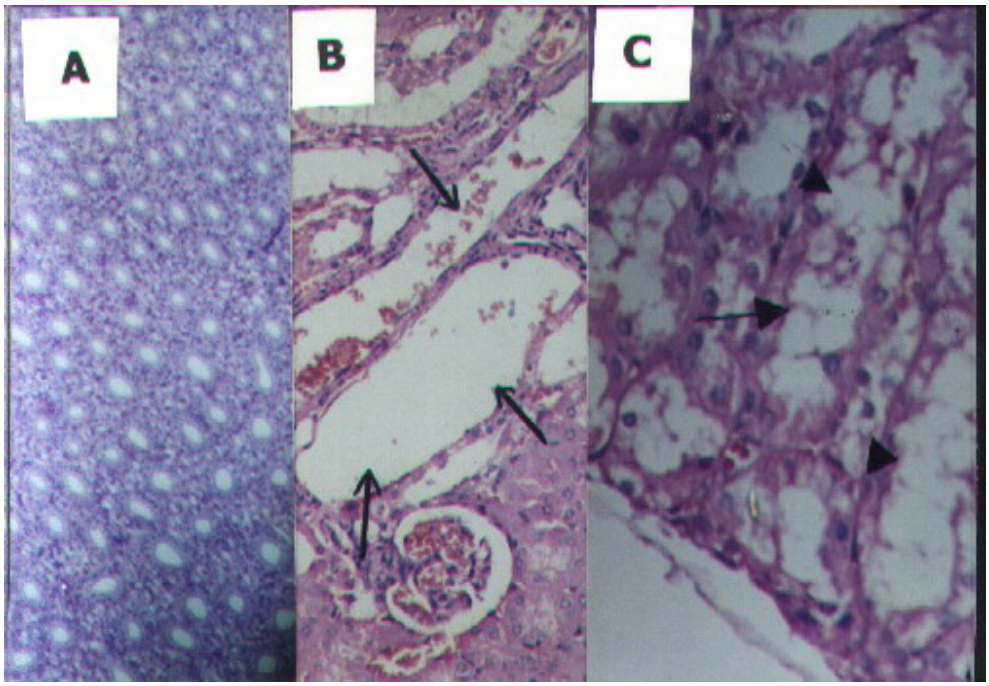

Fig. 3. Histopathological changes in kidneys of albino rats by a single dose of cyto-hepatotoxin $PvHTXII_E$. (A): Control with normal morphology (H & E, x 150), (B) Tubular dilatation (long arrows) (H & E, x 350), (C) Epithelial diaquamation (arrow head), (H & E, x 750).

abnormal levels of chemical analytes, was noted at 24th hour. Levels of urinary urea, osmolality, creatinine, sodium and potassium were also found to be altered, most significantly ($P < 0.01$) at 24th hour (Fig. 2A). In similarity with blood, urinary system exhibited disparity, which was occurred at 12^{th} hour and subsided at 48th hour.

Gradual increase of hepatic enzymes (ALT, AST, ALP and γGT) (Fig. 2 B) level was also noted at 12th hour, presenting significant increase at 24th hour and subsiding after 36 hours. No significant alteration or effect was noted on LDH and CPK enzyme levels.

Histpathological examination

Histopathological studies of kidney tubules and glomerulus in test group show mild to moderate morphological changes at 24th hour (Fig. 3 B, C) as compared to normal groups (Fig. 3A). Dilatation of tubules (3B) and presence of tubular epithelial disquamation (3C) was noted at 24 hours. No significant changes were noted in groups of testing periods 6th, 12th, 36th, and 48th hours.

DISCUSSION

In present study, we describe the nephrotoxic effects of a hepatoxin, $PvHTxII_E$, from contaminated shellfish, *Perna viridis*. Marked impairment of renal function was noted by a single dose of $PvHTxII_E$ in experimental animals causing alteration in osmolality, urea, creatinine, chloride, sodium, potassium, bicarbonate and protein. However, evaluation of results suggested that this is not a direct effect, rather a manifestation occurred during metabolism and elimination of the toxin through renal system, mainly kidney tubules. Disruption of renal function starts at 12th hour which remains impaired, although non-significantly, upto 48 hours. The parameters most significantly altered were osmolality, urea, creatinine, potassium and calcium. Marked impairment of renal function, as indicated by abnormal levels of chemical analytes, was noted at 24th hour. Levels of urinary urea, osmolality, creatinine, sodium and potassium were also found to be altered, most significantly at 24th hour. In similarity with blood, urinary system

exhibited disparity, which was occurred at 12th hour and subsided at 48th hour. Alteration of plasma and urinary osmolality was a significant pointer exhibiting impairment of absorption, distribution and utilization mechanism of macro and micro chemical components present in body fluids. Osmolality represent the collection of glucose, potassium, sodium and urea flowing through cellular and fluid components of the body and presents energy, ionic-channels and metabolic end-product processes of the living system. Alteration of electrolytes, sodium and potassium also of significant importance, resulting in possible impairment of ionic channels causing unnecessary accumulation of macro and microelements in cells or sudden elimination, resulting in cellular disruption and histopathological alteration; as observed in present study in kidney tubules.

Previously done studies suggested that shellfish toxins, such as gonautoxin, are excreted through glomerular filtration (Andrinolo *et al.,* 2002), which cause alteration in creatinine clearance through kidneys. The pathophysiology was described as vascular hypotension, that is responsible for reduced glomerular filtration rate (Andrinolo *et a.,* 2002). A few earlier studies also pointed out related clinical conditions such as hypertension, hepatic failure, altered hemodialysis state and concomitant fatality (Hines *et al.,* 1993; Carmicheal, 1996; Gessner *et al.,* 1997). Moreover, previously, hepatic and renal toxicity of shellfish toxins were also described by several in-depth studies (Fettman *et al.,* 1985; Hooser *et al.,* 1989; Kotak *et al.,* 1996; Alam and Qasim, 1997). Furthermore, cytotoxic response and cellular ion influx by shellfish toxins, was also demonstrated by various methods in animals and cell cultures (Harada *et al.,* 1994; Alam and Qasim, 1997; Morales- Tlalpan and Vaca, 2002).

Nobre *et al.* (1999) reported that induction of microcystin-LR, a toxin obtained from cyano-bacteria, *Microcystis aeruginosa,* in animals cause alteration of kidney function parameters, renal vascular resistance, glomerular filtration rate and urinary flow. They have observed that microcystin-LR impairs the renal function, probably causing glomerular lesions, and promoting renal alteration, either through direct or indirect action (Nobre *et al.,* 2001). In present study, we have noted moderate histopathological changes manifested in tubules and glomerulus, although not probably induced by a direct action of $PvHTxII_E$, but a manifestation of elimination process of toxin through renal system. However, even such a moderate level of histological alteration caused marked impairment of renal function parameters, such as urinary osmolality and urea, and blood potassium levels. Nobre *et al.* (2001) suggested that such type of renal changes occurs probably by damaging both vascular and glomerular sites. Histopathological examination in present study showed epithelial disquamation in tubules, tubular dilatation and infiltration of cellular debris in kidneys after exposure to $PvHTxII_E$, notably at 24th hour. Prior to 24 hours, as well as after 24 hours, no histopathological changes in kidneys was noted in any of the experimental animals, suggesting that elimination of toxins starts probably between 18 to 24 hours after administration of toxin. Moreover, absence of any such histological manifestation at 36 hr to 48 hr also suggests that elimination of toxin, $PvHTxII_E$, from renal system was completed before 36 hours. This reveals a distinguished behavioral characteristic of $PvHTxII_E$, unlike other shellfish toxins, which sometimes take 2 to 8 weeks of elimination period in experimental dosing in oysters (Plakas *et al.,* 2002, 2004).

Alteration of urinary excretion and renal clearance is reported to be one of the clinical signs of shellfish toxicity, which in some cases, tends to form anuria. Andrinolo *et al.* (2002) reported decay in urine excretion in animals, when administered gonyautoxin. Anuria was also noted few hours after toxin administration. It is suggested that when the arterial pressure is reduced, renal flow is also decreased. If the fall is greater than 55%, the mechanism of auto-regulation at the juxta-glomerular level is surpassed and cannot overcome this drastic fall, which leads to anuria. Severe case of anuria has been described in human patients intoxicated with PSP toxin (Montebruno, 1993). Studies performed in experimental animals regarding accumulation and elimination of shellfish toxins also provided useful information related to duration of toxin presence, its metabolism and probable ability to induce clinical and pathological

conditions in living systems (van Dolah and Ramsdell, 2001; Plakas *et al.,* 2002, 2004; Wang *et al.,* 2004). It was noted that elimination of brevet ox in PbTx-1 and PbTx-2, from algal bloom *Karenia brevis,* present in contaminated oysters *(Crassostrea virginica)* takes place within 2 weeks after experimental dosing (Plakas *et al.,* 2004). Moreover, it was also observed that different shellfish toxins have distinct elimination period ranging from 2 to 8 weeks (Plakas *et al.,* 2002).

Alteration of calcium level also plays an important role in enhancing the toxic effect induced by shellfish and algal toxin. We have demonstrated a significant calcium influx after the administration of $PvHTxII_E$ in experimental animals (Alam and Qasim, 1997). Nobre *et al.* (2001) also suggested that the increase in perfusion pressure could be related to alteration in the homeostasis of cellular calcium. Use of indomethacin and dexomethason and their respective protective effect suggest a significant role of prostaglandin and calcium in the alteration triggered by shellfish toxin (Nobre *at aI.,* 2001). Similarly several other previous studies have suggests a major role of extracellular calcium for "maitotoxin" effect in a wide range of cellular preparations (Gusovsky and Daly, 1990; Sorrentino *et al.,* 1997; Escobar *et al.,* 1998). Morales-Tlalpan and Vaca (2002) suggested that maitotoxin effect is potentiated by increments in the intracellular calcium concentration through mediation of non-selective cation channels. It was also suggested that a more complex mechanism, such as calcium calmodulin interaction or calcium-dependent phosphorylation process might be involved in the activation of non-selective cation channels by shellfish toxins (Morales-Tlalpan and Vaca, 2002).

In conclusion, we have described the nephro-toxic effect of a shellfish hepatotoxin, $PvHTxII_E$, after a single dose in experimental animals. The toxin induced marked alteration of renal system, manifested through alteration in kidney function parameters such as urinary osmolality, urea, creatinine and blood potassium levels. However, after thorough assessment of results, it is suggested that the nephrotoxic effect induced by $PvHTxII_E$ is not a direct one, but a patho-physiological manifestation occurred during metabolism and elimination of toxin through renal system.

REFERENCES

ALAM, J.M., JAMAL, Q., IQBAL, N. AND ALAM S.M., 1992. Effects of marine toxins on albino rats. *J. Coll. Phys. Surg. Pakistan*, **2**: 73-78.

ALAM, J.M., ALAM, S.M. AND QASIM, R., 1993. Pathogenesis of the morphological lesions in various organs in guinea pigs produced by cytotoxic compounds from marine animals. *Pakistan J. Path.,* **4**: 95-99.

ALAM, J.M., AMIR, R., QASIM, R., ALAM, S.M. AND KHAN, M.A.J., 1995a. Comparative study of local effects induced by venoms from medically important species of sea-snake *(Microcephalophis gracilis gracilis),* land-snake *(Naja naja naja)* and scorpion *(Vachonus rajasthanicus)* trom Sindh region, Pakistan. *Pakistan J. Zool.,* **27**: 215-217.

ALAM, J.M., AMIR, R., QASIM, R., KHAN, M.A.J. AND ALAM, S.M., 1995b. Comparative studies on low molecular weight cytotoxins from medically important species of sea-snake *(Hydrophis lapemoides, Lapemis curtus)* and scorpion *(Compsobuthus acutecarinatus)* trom Sindh region, Pakistan. *Pakistan J. Zool.,* **27**: 311-315.

ALAM, J.M., QASIM, R. AND ALAM, S.M., 1995c. Effects of drugs against cytotoxic compounds trom marine animals IV-Hepatotoxic activity of sea-snake *Hydrophis cyanocinctus* venom. *Pakistan J. pharmaceut. Sci.,* **8**: 63-68.

ALAM, J.M. AND QASIM, R, 1997. Isolation of cytotoxic compounds from marine animals. I-Hepatotoxin PvHTxlIE from Shellfish *Perna viridis* (Green mussel). *Pakistan J. Zool.,* **29**: 61-75.

AMMONS, D. AND SAMPAYO, M.A.M., 2001. Domoic acid in Portuguese shellfish and fish. *Toxicon,* **39**: 893-904.

ANDRINOLO, D., IGLESIA, V., GARCIA, C. AND LAGOS, N., 2002. Toxicokinetics ands toxicodynamics of gonyautoxins after an oral dose in cats. *Toxicon,* **4069:** 699-710.

BURNETT, J.W. AND CALTON, G.I, 1977. Review Article: The chemistry and toxicology of some venomous pelagic coelenterates. *Toxicon,* **15**: 177-196.

CARMICHAEL, W.W., 1996. Liver failure and human deaths at hemodialysis center in Brazil: microcystins as a major contributing factor. *Harmful Algal Blooms,* **15** IOC UNESCO, Rains p. ll

CHEN, C.Y. AND CHOU, R.N., 2001. Accumulation and depuration of paralytic shellfish poisoning toxins by purple clam *Hiatula rostrata* Lighttot. *Toxicon,* **39**: 1029-1034.

CHONG, M.W.K, WONG, B.S.F., LAM, P.KS., SHAW, G.R AND SEAWRIGHT, A.A., 2002. Toxicity and uptake mechanism of cylindrosermopsin and lophoprotomin in primary rat hepatocytes. *Toxicon,* **40**: 205-212.

ESCOBAR, L.I., SALVADOR, C., MARTINEZ, M. AND VACA, L., 1998. Maitotoxin, a cationic channel activator. *Neurobiology,* **6**: 59-74.

FETTMAN, M.I, ALLEN, T.A., WILKER, W.L., RADIN, M.I AND EUBANK, M.C., 1985. Single injection method

for evaluation of renal function with 14C-inulin and 3H-tetra ethylammonium bromide in dogs and cats. *Am. J. vet. Res.,* **46**: 482-485.

FLANAGAN, A.F., CALLANAN, KR, DONLON, I, PALMER, R, FORDE, A. AND KANE, M., 2001. A cytotoxicity assay for the detection and differentiation of two families of shellfish toxins. *Toxicon,* **39**: 1021-1028.

FREITAS de MAGALHAES, V., SOARES, R.M. AND AZEVEDO, S.M.F.O., 2001. Microcystin contamination in fish from the Jaeanepagua Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risks. *Toxicon,* **39**: 1077-1086.

GESSNER, B.D., BELL, P., DOUCETTE, G.-I, MOCZYLLOWSKI, E., POLI, M.A., DOLAH, F.Y. AND HALL, S. 1997. Hypertension and identification of toxin in human urine and serum following a cluster of mussel-associated paralytic shellfish poisoning outbreaks. *Toxicon,* **35**: 711-22

GUSOVSKY, F. AND DALY, J, 1990. Maitotoxin: a unique pharmacological tool for research on calcium-dependent mechanisms. *Biochem. Pharmacol.,* **39**: 1633-1639.

HARADA, K.-L., OHTANI, 1., IWAMOTO, K., SUZUKI, M., WATANABE, M.F., WATANABE, M. AND TERAO, K., 1994. Isolation of cylindrospermopsin from a cyanobacterium, *Umezakia natans* and its screening methods. *Toxicon,* **32**: 73-84.

HINES, H.B., NASEEM, S.M. AND WANNEMACHER, Jr. R.W., 1993. ^{3}H-Saxitoxinol metabolism and elimination in the rat. *Toxicon,* 31: 905-908.

HOOSER, S.B., BEASLEY, Y.R, LOVELL, RA., CARMICHAEL, W.W. AND HASCHEK, W.M., 1989. Toxicity of microcystin-LR, a cyclic heptapeptide hepatotoxin from *Microcystis aeruginosa*, to rats and mice. *Vet. Pathol.,* **26***:* 246-252.

ITO, E., SATAKE, M., OFUJI, K., HIGASHI, M., HARIGAYA, K., McMAHON, T. AND YASUMOTO, T., 2002. Chronic effects in mice caused by oral administration of sublethal doses of azaspiracid, a new marine toxin isolated from mussel. *Toxicon,* **40**: 193-204.

KOTAK, B.G., SEMALULU, S., FRITZ, D.L., PREP AS, E.E., HRUDEY, S.E. AND LOPPOCK, RW., 1996. Hepatic and renal pathology of intraperitoneally administered microcystin-LR in Rainbow Trout *(Oncorhynchus mykiss). Toxicon,* **34**: 517-525.

MEHTO, P., ANKELO, M., HINKKANEN, A., MIKKAILOV, A., ERIKSSON, J.E., SPOOF, L. AND MERILUOTO, J, 2001. A time-resolved flouro-immunometric assay for the detection of microcystins, cyanobacterial peptide hepatotoxins. *Toxicon,* **39**: 831-836.

MONTEBRUNO, D.Z., 1993. Anatomo-pathologic study of paralytic shellfish intoxication in the XII region of Chile. *Rev. Med. Chile (Chile),* **121**: 94-97.

MORALES-TLALPAN, Y. AND VACA, L., 2002. Modulation of maitotoxin response by intracellular and extracellular cations. *Toxicon,* **40**: 493-500.

NOBRE, AC.L., JORGE, M.C.M., MENEZES, D.E., FONTELES, M.C. and MONTEIRO, H.S.A, 1999. Effect of microcystin-LR in isolated perfused rat kidneys. *Brazilian J. med. biol. Res.,* **32**: 985-988.

NOBRE, AC.L., COELHO, G.R, COUTINHO, M.C.M. SILVA, M.M.M., ANGELIS, E.Y., MENEZES, D.E., FONTELES, M.C. AND MONTEIRO, H.S.A, 2001. The role of phospholipase A2 and cyclo-oxygenase in renal toxicity induced by microcystin-LR. *Toxicon,* **39**: 721-724.

NORRIS, R.L.G., SEAWRIGHT, A.A., SHAW, G.R., SENOGLES, P., EAGLESHAW, G.K, SMITH, M.J., CRISWELL, RK AND MOORE, M.R, 2002. Hepatic xenobiotic metabolism of cylindrospermopsin in vivo in the mouse. *Toxicon*, **40**: 471-76.

PARK, H, NAMIKOSRI, M., BRITTAIN, S.M., CARMICHAEL, W.W. AND MURPHY, T., 2001. [D-Ieul] microcystin-LR, a new microcystin isolated from water bloom in a Canadian prairie lake. *Toxicon,* **3**: 855-862.

PLAKAS, S.M., EL SAID, KR, JESTER, E.L.E., GRANADE, H.R, MUSSER, S.M. AND DICKEY, R.W., 2002. Confirmation of brevetoxin metabolism in the Eastern oyster *(Crassostrea virginica)* by controlled experiments to pure toxins and *Karenia brevis. Toxicon,* **40:** 721-729.

PLAKAS, S.M., WANG, Z., EL SAID, KR, JESTER, E.L.E., GRANADE, HR, FLEWELLING, L., SCOTT, P. AND DICKEY, RW., 2004. Brevetoxin metabolism and elimination in the Eastern oyster *(Crassostrea virginica)* after controlled exposure to *Karenia brevis. Toxicon,* **44:** 677-685

RADBERGH, C.M.I., BYLUND, G. AND ERIKSSON, J.E., 1991. Histological effects of microcystin-LR A cyclic peptide toxin from the cyanobacterium (Blue-green algae) *Microcystis aeruginosa,* on common carp *(Cyprinus carpio* L.). *Aquat. Toxicol.,* **20**: 131-146.

RAMSTAD, H., HORGAARD, P., YASUMOTO, T., LARSEN, S. AND ANNE, T., 2001. Monthly variations in diarrhetic toxins and yessotoxin in shellfish from coast to inner part of the Sognefjond, Norway. *Toxicon,* **39**: 1035-44.

ROSSINI, G.P., SGARBI, N. AND MALAGUTI, C., 2001. The toxic responses induced by okadoic acid involve processing of multiple caspase isoforms. *Toxicon,* **39**: 763-770.

RUSSELL, F.E., 1984. Marine toxins and venomous and poisonous marine animals. In: *Advances in marine biology* (eds. J.R.S. Baxter, F.S. Russell and C.M. Yonge). Pergamon Press, London.

SATO, S., OGOT A, T., BORJA, Y, GONZALES, c., FUKUYO, Y and KODAMA, M., 2000. Frequent occurrence of paralytic shellfish poisoning toxins as dominant in marine puffer from Tropical water. *Toxicon,* **38**: 1101-1110.

SHIMIZU, Y, 1978. Dinoflagellates toxins. In: *Marine natural products* (ed. P. Scheuer), vol I., pp. 1-41. Pergamon Press, Oxford.

SORRENTINO, G., MONSURRO, M.R., SINGH, I.N. and KANFER, IN., 1997. Membrane depolarization in LA-N-l cells. The effect of maitotoxin is Ca (2+) and Na

(+)-dependent. *Mol. chem. Neuropath., **30**: 199-211.*

SILKKER, W.I, SCALLET, AC. and GAYLOR, D.W., 1998. Biologically-based dose-response model for neurotoxicity risk assessment. *Toxicol. Lett.,* **102-103**: 429-33.

TERAO, K., ITO, E., SAKAMAKI, Y, IGARASHI, K., YOKOYAMA, A. AND YASUMOTO, T., 1988. Histopathological studies of experimental marine toxin poisoning. II-The acute effects of maitotoxin on the stomach, heart and lymphoid tissues in mice and rats. *Toxicon,* **26**: 395-402.

TERAO, K., ITO, E., MURAKAMI, M. AND YUAMAGUCHI, K., 1989. Histopathological studies of experimental marine toxin poisoning III-Morphological changes in the liver and thymus of male ICR mice induced by Goniodomin A, isolated from the dinoflagellate *Goniodoma pseudogoniaulax. Toxicon,* 27: 269-271.

TERAO, K., ITO, E., YASUMOTO, Y and YAMAGICHI, K., 1990. Enterotoxic, hepatotoxic and immunotoxic effects of dinoflagellate toxins on mice. In: *Toxic marine phytoplankton* (eds. E. Graneli, B. Sundstrome, L. Edler and D.M. Anderson), pp. 418-423. Elsevier, New York.

TERAO, K., ORMORI, S., IGARASHI, K., OHTANI, I., WATANABE, M.F., HARADA, K.I., ITO, E. AND WATANABE, M., 1994. Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated trom blue-green alga *Umezkia natans. Toxicon,* **32:** 833-843.

VALE, P. AND SAMPAYO, M.A.M., 2001. Domoic acid in Portuguese shellfish and fish. *Toxicon,* **39:** 893-904.

VAN DOLAH, F.M. AND RAMSDELL, J.S., 2001. Review and assessment of *in vitro* detection method for algal toxins. *J. AOAC Int*., **84**: 1617-1625.

WANG, Z., PLAKAS, S.M., EL SAID, KR, JESTER, E.L.E., GRANADE, H.R. AND DICKEY, R.W., 2004. LC/MS analysis of brevetoxin metabolites in the Easterr oyster *(Crassostrea virginica). Toxicon,* **43:** 455-465.

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