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Research Article

TOXICITY BIOASSAY AND SEMI-QUANTITATIVE HISTOPATHOLOGICAL CHANGES IN THE GILLS OF PADDY FIELD CRAB, *PARATELPHUSA HYDRODROMUS* (HERBST) EXPOSED TO ARSENIC

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ABSTRACT

Fresh water paddy field crab *Paratelphusa hydrodromus* were exposed to sub-lethal concentration of arsenic (22.8 ppm) (1/5th of LC_{50} concentration) for 72 hours along with 48 hours recovery of 72 hrs exposed animals to toxicant free water. The gill morphological alterations were examined by light microscope using semi-quantitative histopathological evaluation. The morphometric measurements were collected from the five different points and examined the width of basal septum, lamellae, apical protuberance in both control and treated organisms exposed to arsenic. Light microscopic examination of the toxicant treated gill revealed serious gross morphological alterations against control animals and were time dependent. The gill after 2 hrs of exposure showed enlargement of branchial septum, lamellae and apical protuberance. The gills fixed after 24 hrs of arsenic exposure showed alteration in the haemolymph flow pattern, haemocyte infiltration and enlargement of branchial septum. In 24 and 48 hrs exposure, pilaster bridging in the lamellae were almost normal and brachial podocyte distribution was dense in the anterior region and gradually decrease towards the posterior edges and leaving large haemal space. Extensive infiltration of haemocytes and focal necrosis was observed in the few lamellae along with clumping of haemocytes and epithelial hyperplasia. During 48 hrs recovery treated animals showed enlargement of lamellae with sub-epithelial edema at the apical protuberances region. The animals transferred to toxicant free water showed quick changes like enlargement of lamellae and branchial septum. Disappearance of pilaster cells and lifting of cuticle from epithelial layer were also noticed during the recovery phase.

INTRODUCTION

Arsenic is a naturally occurring element which has many positive agricultural applications in contrast to its biological toxicity and threat to wildlife. Arsenicals are used in agriculture as feed additives, herbicides, insecticides, and cotton defoliants. The arsenic cycle involves both biotic and abiotic reactions. Various plants, invertebrates and microorganisms play a major role in the transformation and movement of arsenicals in soil and water.

Arsenic, primarily in the inorganic form, is present in the earth's crust at an average of 2-5 mg kg^{-1}. The mean concentration of arsenic in igneous rocks, 1.8 mg kg^{-1}; and sandstones, 1.0 mg kg^{-1} Sedimentary rocks range from 0.1 mg kg^{-1} to as high as 2900 mg kg^{-1} arsenic. Arsenite and arsenate are weathered from arsenic-containing rocks which are considered the major natural source of arsenic, estimated to release 45, 000 metric tons/year (Knowles and Benson, 1983). The presence of arsenic (As) in water and its effect on human health through both drinking and agricultural practices is of serious concern over worldwide. Freshwater ecosystems exhibit a high natural variability in their physical and chemical properties due to local differences in geology and climate. Hence, they are more susceptible to anthropogenic influences than the more consistent and stable marine environments (Rainbow and Dallinger, 1993). Consequently; both the quality and quantity of water are affected by an increase in anthropogenic activities (Sanders, 1997; Sharma and Agrawal, 2005; Lavanya *et al.,* 2011; Kovendan *et al.,* 2013; Tao, 2014).

Aquatic organisms accumulate As in their bodies through food chain, water and sediments (Rahman, Hasegawa and Lim., 2012). The environmental contamination extends it residual effects in wide varieties of processed food (Kulkarni, Venkateshvaran, and Wavde, 2005). The higher concentrations of inorganic arsenic were found in lemon sole (*Microstomus Kitt*) composite (19.29 mg/kg) and skate (*Raja spp)* composite (20.17 mg/kg). According to other results, the levels of total arsenic in skate are typically high; with a range of 14.4-61.5 mg/kg. Similarly in canned crab food the incidence of inorganic arsenic were reports as 9.16 mg/kg (Kulkarni,

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Venkateshvaran, and Wavde, 2005; Food Additives and Contaminants, 2000). Many of the earlier studies have demonstrated that arsenic compounds are toxic metalloid of the environment and severely affects the normal physiology of crustaceans. The present study was aimed to demonstrate the toxicity and histopathaological effects of arsenic in fresh water paddy field crab *Paratelphusa hydrodromus.,* an ideal candidate for the fresh water system being comparatively long living and shows minimum local migration.

MATERIAL AND METHODS

Healthy specimens of *Paratelphusa hydrodromus* (Stage-4, intermolt[6]) were collected from the paddy field Vellayani region located 11km South of Thiruvananthapuram, Kerala. Animals were brought to laboratory and acclimated at 27°C, 14L: 10D photo period, in 50L glass tank for four days. Stock solution of arsenic (1000ppm) was prepared by dissolving AR grade 4.165gm sodium arsenate (Na_2H AsO_4 $7H_2O$) in double distilled water and sub-dilutions were made from the stock by adding the water collected from the respective habitat. The important physio-chemical characteristics of dilution water were determined (mean temperature-27°C, pH-7.2, dissolved oxygen-6.8mg/L total suspended solids-165.7mg/L, hardness-19.25mg/L, $CaCO_3$ and Arsenic-26ppb).

Acute toxicity assay (LC_{50}) for 24, 48, 72 and 96 hrs was conducted following the standard protocol described by Litchfield and Wilcoxon., 1947. Fresh water paddy field crab *Paratelphusa hydrodromus* (N=40) were exposed to sub lethal concentration of arsenic 22.8 ppm ($1/5^{th}$ of LC_{50}) concentration for a period of 72 hrs. Exposed animals were sampled at different time intervals of control, 2, 24, 72 and 48 hrs, following recoveries after transferring exposed animals to toxicant free water. Five crabs were sampled and first gill from each side were dissected and fixed in 10% neutral buffered formalin (NBF). The fixed gills were then dehydrated, cleared in chloroform and embedded in paraffin wax with ceresin at 60°C. Section were cut at 6µm thickness by using Rotary micro tome and stained with Harris Haematoxylin and Eosin (H&E). Semi-quantitative evaluation of arsenic effect was performed by random selection of five representative locations and pooled histological effect during toxic and recovery phase.

RESULTS

The static toxicity bioassay values (LC_{50}) for 24, 48, 72 and 96hrs were 136, 128, 121.5 and 114 ppm respectively and 72 hrs $1/5^{th}$ of LC_{50} concentration (22.8 ppm) were selected for studying histological impact of arsenic in paddy field crab, *Paratelphusa hydrodromus* (Shibu Vardhanan, and Tresa Radhakrishnan, 2002).

addy field crab exposed to 22.8 ppm arsenic showed progressive changes in the gill histology and was time and dose dependent. The gills of *P. hydrodromus* was typical phyllobranchialte (lamellar) gill structure as illustrated in control photomicrograph (Table 2 and Fig. 1). The phyllobranchiate structure with central stem (aixs/raphae) that bears serially arranged paired plates or leaf like lamellae, which are actually flattened sac with a protuberance in the apical region. The lamellae, with are flattened sac, composed of double layer of cuticle enclosing a thin layer of epithelial cells (Table 2 and Fig. 1). These cellular layers regularly connected by many columnar supporting cells (pilaster cells) arising from the epithelial sheets and joining the two surfaces of a lamella across haemolyph space (Table 2 and Fig. 1).

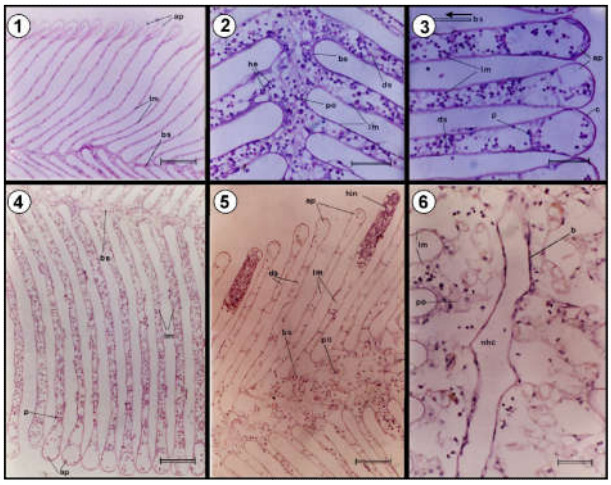

Fig 1 Gill of normal crab showing Phyllobrachiate structure (Bar = 140 µm)
Fig.2-3 Gill of 2 hours arsenic exposed crab (Bar= 60 µm)
Fig. 4 Gill of 24 hours arsenic exposed crab (Bar= 50 µm)
Fig. 5-6 Gill of 48 hours arsenic exposed crab (Bar= 50 µm)

Summary of semi-quantitative response of gill exposed to 22.8 ppm arsenic was tabulated and presented in the Table 2. The gill fixed after 2 hours of exposure showed enlargement of branchial septum and lamellae (Table 2 and Fig 2). Apical protuberance was enlarged due to the toxicant exposure. Twenty four hours arsenic exposed gill showed uneven haemocyte distribution in branchial septum and increased incidence of podocytes (Table 2 and Fig. 3). Pilaster bridging and excretory cells distribution (podocytes) are almost similar both in the treated and control animals (Fig. 4). Increased incidence of haemocyte infiltration along with change in the haemolyph flow pattern was noticed in the 48 hrs exposed gill (Table 2 and Fig. 5). Branchial podocyte distribution was dense in the anterior region and gradually decreased towards the posterior region and leaving large haemal space (Table 2 and Fig. 6, 7).

Table 1 Data showing 24, 48, 72 and 96hr LC_{16}, LC_{50} and LC_{84}, of *P. hydrodromus* exposed to arsenic along with slope function and their 95% confidence limits.

Time (hr)	LC_{16} (ppm)	LC_{50} (ppm)	LC_{84} (ppm)	95% confidence limit (ppm)	Slope	95% confidence limit (ppm)
			Arsenic			
24	120.0	136.0	164.0	128.30-144.20	1.17	1.03-1.33
48	116.0	128.0	143.0	123.10-133.14	1.11	1.03-1.33
72	104.0	121.5	135.0	115.64-127.65	1.14	1.03-1.27
96	101.8	114.0	127.0	108.79-119.46	1.12	1.04-1.16

Table 2 semi-quantitative spread sheet showing the histopathological response in the gill of *P. hydrodromus* exposed to 22.8 ppm arsenic.

	Exposure time in hours (hrs)				
Width in µ	Control	2	24	72	48hrs Recovery
Lamella	ok 6-11	bu 46-49	bu 30-39	lc** 6-8	bu** 26-70
Basal septum	ok 21-26	di 67-72	di* 272-281	di 211-219	di** 52-379
Apical protuberance	ok 45-57	s 74-77	s 42-59	s* 76-76	s** 52-379 r
Haemocytes	ok	almost ok	few in, che	che**, fne, few in	in, fe**
Pilaster- cells	ok	ok, br	few bu	mostly a	de**
Haemal canal	ok	almost ok	nhc	bh **	nhc
Epithelium	ok	Almost ok	few nhc fne	ehy few chy	tep, sed
Podocytes	ok	ind	agg*	agg***	Agg**
	* * Medium,	** High,	***Very high R		

The gill fixed after 72 hrs of arsenic exposure showed very prominent morphological changes and the results was tabulated and presented (Table 2 and Fig 8-11). Enlargement of branchial septum was noticed along with infiltration of haemocytes and focal necrosis at few places (Table 2 and Fig.9, 10, 11). Gill lamellae showed lateral compression and thereby blocking of haemal flow at several regions towards apical protuberance and basal septum (Fig.9, 10, 11).

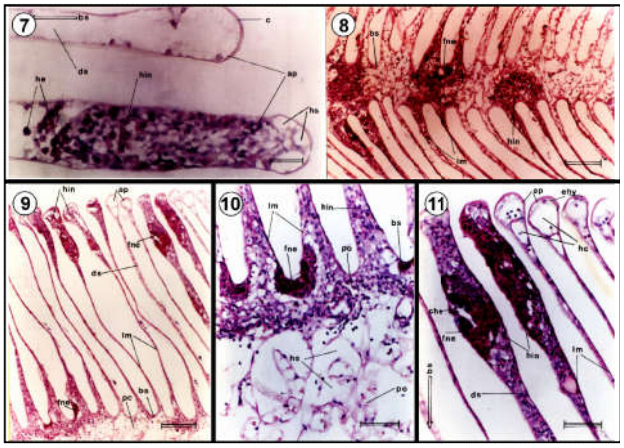

Fig 7-11 Gill of 72 hours arsenic exposed crab (Bar= 50 µm).

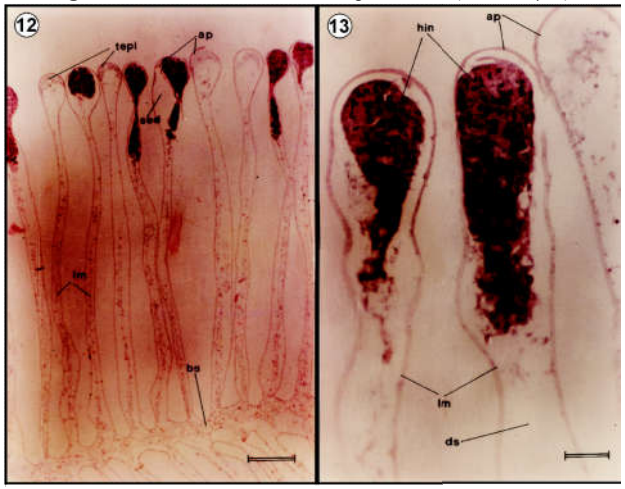

Fig 12-13 Gill of crab recovering from arsenic exposure (72 hours post treatment)

Extensive infiltration of haemocytes, epithelial hyoerplasia and focal necrosis leads normal haemal flow pattern (Table 2 and Fig.9, 10, 11). After 72 hours of treatment, animals were transferred to clean water in order to study the speed with capability to recover from the toxic effect of the arsenic. A recovery period of 48 hours showed notable characteristics in gill morphology (Table 2 and Fig. 12 and 13). Dilation of lamellae and branchial septum was observed along with extra accumulation of haemolymph and edema (Table 2 and Fig. 12 and 13). Lifting of cuticle from epithelium was one of the notable features along with disappearance of pilaster cells (Table 2 and Fig. 12 and 13). Thickening of epithelial layer was observed in few filaments (Table 2 and Fig. 13).

DISCUSSION

It has been considered that most of the edible crabs are an important, proteinaceous and tasty food consumed over worldwide by mankind. Aquatic organisms surviving in most of the water bodies are prone to severe metal contaminations from various sources. These facts, posed us to study the toxicity of the metalloid arsenic compounds. Our earlier observations of arsenic exposure to paddy field crab, *P. hydrodromus* for different time intervals such as 24, 48, 72 and 96 hours exposed at 136, 126, 121.5 and 114 ppm respectively were reported (Shibu Vardhanan, and Tresa Radhakrishnan, 2002). The median toxicity values were rather high in case of decapods and fishes and showed wide differences between species (NRCC, 1978). The populations of fresh-water zooplankton and insects get reduced when they are exposed to a range of 2.2 to 11.1 ppm sodium arsenate. Similarly, an arsenic concentration of 0.52 ppm resulted in 16 % decrease in reproduction of *Daphnia magna* (Weis, Weis and Coohil, 1991). The crab *Uca puligator* exposed to CCA (Chromated Copper Arsenate) reduced regeneration power (WHO 1989).It was evidenced that the level of arsenic in the different body parts of crab, *Barytelphusa guerini* of Godavari river system were higher than the recommended standards in food (Sayyad *et al* ., 2007). Similarly in tropical condition the reported 96h LC_{50} values of arsenic as 40 ppm for fish, *Etroplus maculates* (Bloch.), 23ppm for clam, *Villorita cyprinoides var cochinensis* (Hanley) and 11 ppm for prawn, *Macrohrachium idella idella* (Fernandez, Tresa, George Thomas and Shibu Vardhanan, 1996).

The toxicity bioassays of arsenic using freshwater chichlid fish *Tilapia mossambica*, concentration up to 15 ppm was found to be non-lethal. Fishes started dying when concentration was increased to 15, 22.5 and 30 ppm, 100% mortality was not experienced in any of the concentration during 96 hrs **(**WHO 1989). These results show relative tolerance of arsenic toxicity to animals.

The gill of paddy field crab function as a complex organ, that serveis many functions like osmotic control, respiration, excretion and probably in storage of waste materials (Couch, 1978). In the present study, paddy crab exposed to 104ppm arsenic showed histopathological changes in the gills and the toxic response was time dependent. Recent study conducted in blue swimmer crab, *Portunus pelagicus,* collected from Persian Gulf waters of Iran, showed bioaccumulation of arsenic in soft tissues of the crab during different seasons (Shahrzad Khoramnejadian and Forouzan Fatemi, 2015).

The gill examined after 72 hrs of exposure showed pathological changes particularly specific to the toxicant, arsenic. Enlargement of branchial septum with infiltration of haemocytes and focal necrosis at few place were already reported in estuarine prawn *Macrobrachium idella idella* **(**Mammachan, 1992). Coagulation of haemocyte in the brachial septum blocks the normal haemal pattern which was very unique to arsenic toxicity **(**Mammachan, 1992). Arsenic toxicity in higher organisms such as fish, salmon *Salmo salar* the sub-cellular toxic effects cause the formation of cytoplasmic protrusion and apoptosis **(**Buchman *et al.,* 1993**)**. Similar reports were evidenced on *Oreochromis mossambicus* gills as affected by the toxicity of arsenic (Ahmed *et al.,* 2013).

Haemocytes infiltration leads to local aggregation of haemocytes at the apical protuberance and results black pigmentation **(**Rinaldo, and Yevich, 1974). This intern results haemocytes free areas and staining affinity were also considerably reduced **(**Rinaldo, and Yevich, 1974). A black spot disease in crustacean has been widely reported and is known to be caused by variety of biological and chemical agents (Couch, 1978; Rinaldo, and Yevich 1974; Lighter and Fontaine 1975; Lighter and Redman, 1977). Scanning Electron Microscopic (SEM) examination of nickel exposed gill of estuarine crab *Scylla serrata* showed collapse of adjacent gill lamellae and enlargement of apical protuberance (Nonnotte, Boitel and Truchot, 1993; Jayakumari, 1995). Recent study conducted on the mudcrab, *Scylla serrata* exposed to arsenic exhibited an appearance of selected abnormal behavioural manifestation including tendency of avoidance, hypersecretion of mucoid element and release of excess excretory products (Sanjib Saha, Mitali Ray and Sajal Ray., 2010). It has been observed that the arsenic exposure resulted with a dose dependent decrease.

CONCLUSION

The present study reveals that fresh water paddy field crab *Paratelphusa hydrodromus* is an ideal biomarker for studying the impact of various environmental contaminations related to agricultural fields. The result of recovery studies reveals animal's tendency and capability to restore from toxic contaminated environment within a very brief time of 48hrs.

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