THREATS OF ARSENIC ON MORPHOLOGICAL SHIFT OF HEMOCYTES OF MOLLUSCS: A HINT TOWARDS ARSENIC-INDUCED CELL DEATH

SRIKANTA GURIA

Immune system is strongly influenced by environmental conditions. Arsenic is an important environmental pollutant. Due to high distribution worldwide, the molluscs accumulate large amount of pollutants, which is responsible for immune challenge. Cellular response in molluscs is carried out by hemocytes that can kill microbes through phagocytosis and cytotoxic reactions. In the present study significant changes were observed in the cytomorphology of hemocytes of aquatic molluscs (*Pila* sp.) upon treatment with arsenic. Treated cells showed different phases of cellular death like formation of membrane blebs, rupture of plasma membrane. Small cytoplasmic vacuoles were found in arsenic (As) treated hemocytes. This report has suggested nature of cell death may be used as an effective biomarker of aquatic pollution. Hemocytes thus serve as a unique tool for the testing of the toxicity of environmental pollutant.

Hemocytes will help to establish an effective bio-indicator which may provide good systems to evaluate the toxic effects of environmental contaminants.

Keywords: hemocytes, molluscs, cellular death, arsenic (As).

INTRODUCTION

There is a wide distribution of arsenic (As) in the environment. Over the years, several epidemiological and scientific reports have revealed the adverse effects of arsenic in plants, animal and humans (Tseng, 1977; Wang *et al*., 2002). Arsenic is an important environmental pollutant, whose risk of poisoning in humans is a public health issue worldwide (Rossman, 2003). Several scientific data suggest that the inorganic forms of arsenic (As) exhibit the highest toxicity level, while organo-arsenicals are usually less toxic (Duker *et al*., 2005). This article discusses effect of arsenic and its role on cytomorphology and death of hemocytes. Aquatic invertebrates have ecological, nutritional significance (Ray *et al*., 2015). Recent evidences have shown that the contamination of freshwater and marine ecosystem by various pollutants produces threat to the natural habitats for invertebrates (Ray *et al.*, 2015).

Freshwater molluscs like *Pila* sp. (Mollusca: Gastropoda) are the vital aquatic resources of India (Subba & Dey., 1989; Prabhakar *et al*., 2009). Hemocytes, the

principal immune effector cells of molluscs (Humphries & Yoshino, 2003), execute varied immunological functions like phagocytosis (Cheng, 1977; Hillyer *et al*., 2003), cytotoxicity (Adema *et al*., 1991; Bogdan, 2001), aggregation (Chen & Bayne, 1995) and encapsulation (Humphries & Yoshino, 2003). By considering the important role of hemocytes in molluscs, it has been decided to study hematological analysis of freshwater species *Pila* sp. at cellular level.

MATERIAL AND METHODS

Laboratory maintenance and acclimatization

42 specimens of *Pila* sp. were manually collected from the selected natural habitats of West Bengal, India. The specimens were transported to the laboratory and maintained the ambient temperature of 25–30°C.

Treatment

Pila specimens (Mollusca: Gastropoda) were maintained in two separate aquariums. One group of *Pila* specimens was maintained in aquarium containing 0.025 mg/L of (sodium arsenite) $NaAsO_2$ for ten days (Nath *et al*., 2012; Guria *et al*., 2016). Control specimens were untreated by any toxic metals.

Collection of hemolymph

Hemolymph was collected aseptically by shell puncture method (Renwrantz *et al*., 1981) and from the heart of *Pila sp* (Ray *et al*., 2013 a,b). The total hemocyte count was determined by hemocytometer under light microscope.

Staining of hemocytes

Hemolymph was placed and smeared directly on sterilized glass slides and were stained by Giemsa, Methylene Blue, and observed under light microscope.

Trypan Blue dye exclusion test

Cells were treated with 50 µl of 0.25% Trypan blue dye solution for 5 minutes and mortality index was calculated (Guria *et al*., 2016).

$$\text{Mortality index} = \frac{\text{Number of cells with blue stained cytoplasm}}{\text{Total number of cells}} \times 100$$

RESULTS

Normal cytomorphological profile of hemocyte of *Pila*

Normal cell morphotypes were noticed in control molluscs.

Effect of toxic metal on cell-structure of haemocytes

Significant changes were observed in the cytomorphology of hemocytes when compared with the control group. Higher magnification of treated cells showed different phases of cellular death like formation of membrane blebs (Fig. 1A) and rupture of plasma membrane (Fig. 1B).

Small cytoplasmic vacuoles found in arsenic (As) treated hemocytes (Fig. 2A). Normal ultra structural morphology was predominately found in the control cells, showing a well-defined plasma membrane and intact nucleus. A significant percentage of hemocytes become pyknotic in treated condition (Fig. 2B).

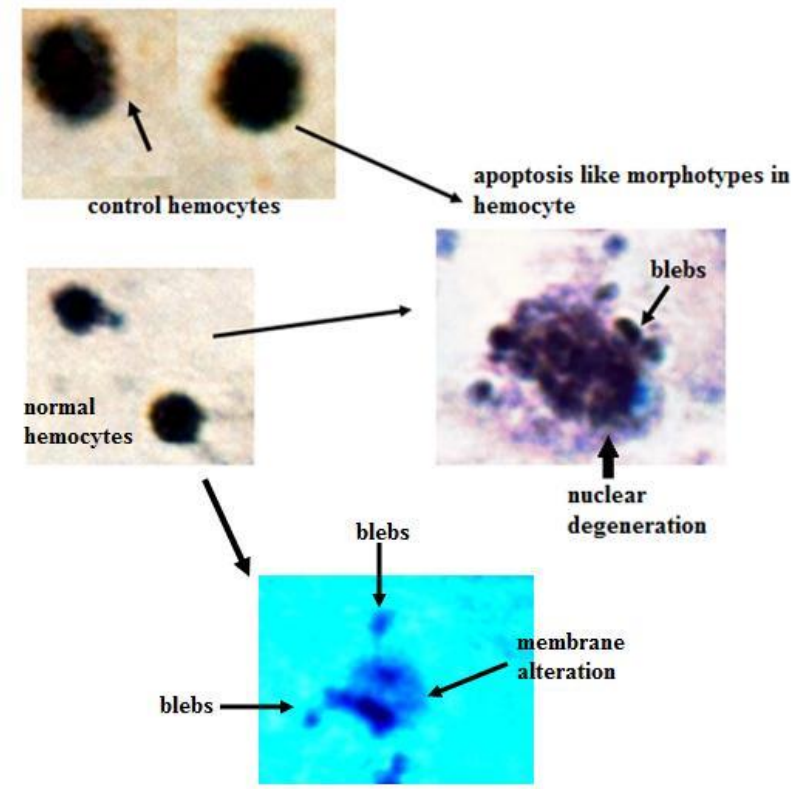

Fig. 1A. Giemsa stained sodium arsenite treated cells showing altered cell surface indicating cellular apoptosis (× 400).

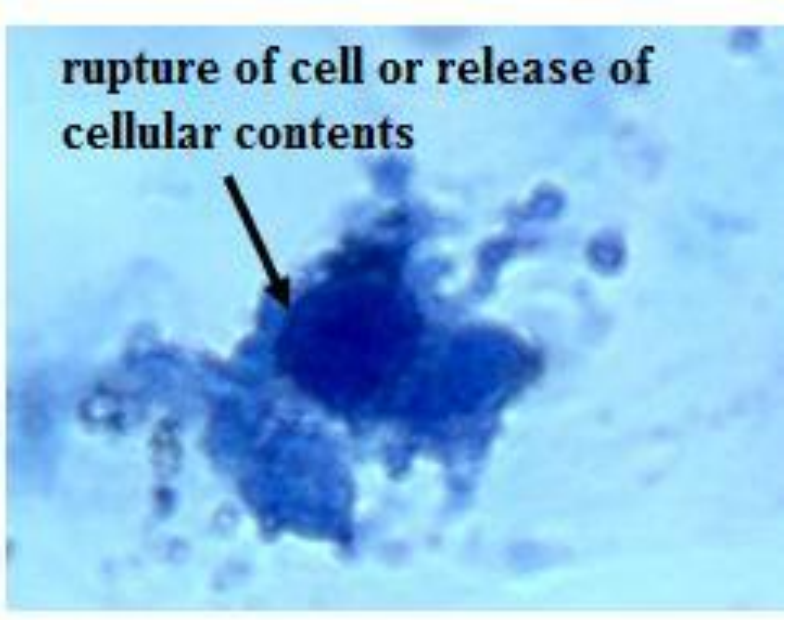

Fig. 1B. Rupture of plasma membrane of treated hemocytes (× 400).

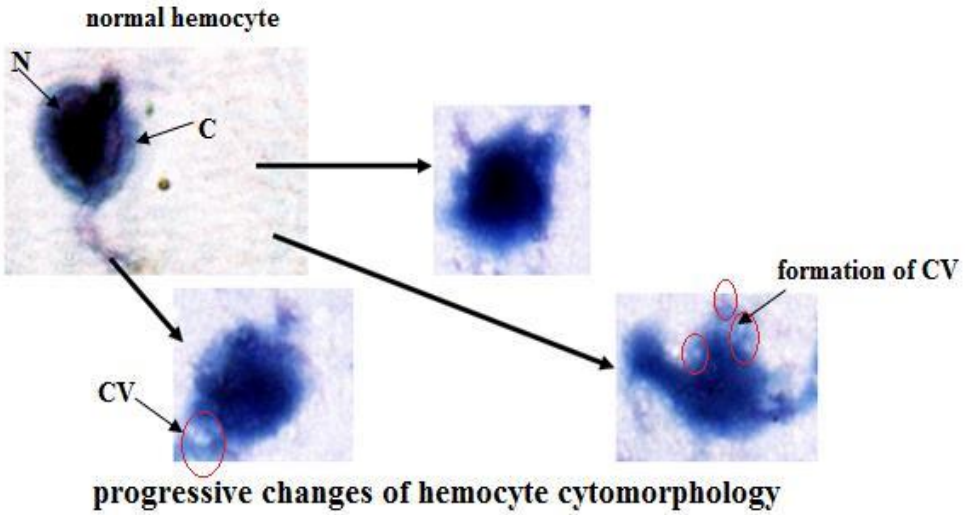

Fig. 2A. Control cells showing a well-defined plasma membrane and intact nucleus.
After arsenic treatment, the cell cytoplasm displayed vacuoles
(N = nucleus; C = cytoplasm; CV = cytoplasmic vacuole) (× 400).

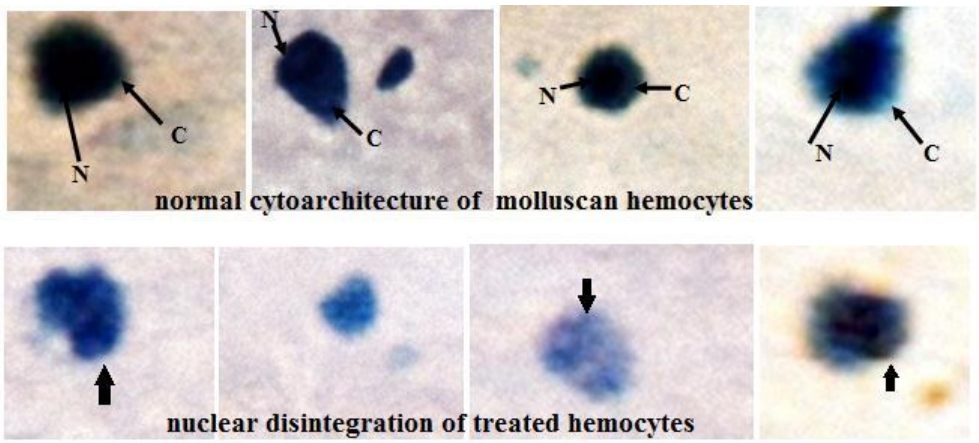

Fig. 2B. Normal cell architecture and deformed cell structure.
Note morphological aberrations of hemocytes due to arsenic treatment
(N = nucleus; C = cytoplasm) (× 400).

Calculation of mortality index

Mean mortality index was significantly increased in treated group (Fig. 3).

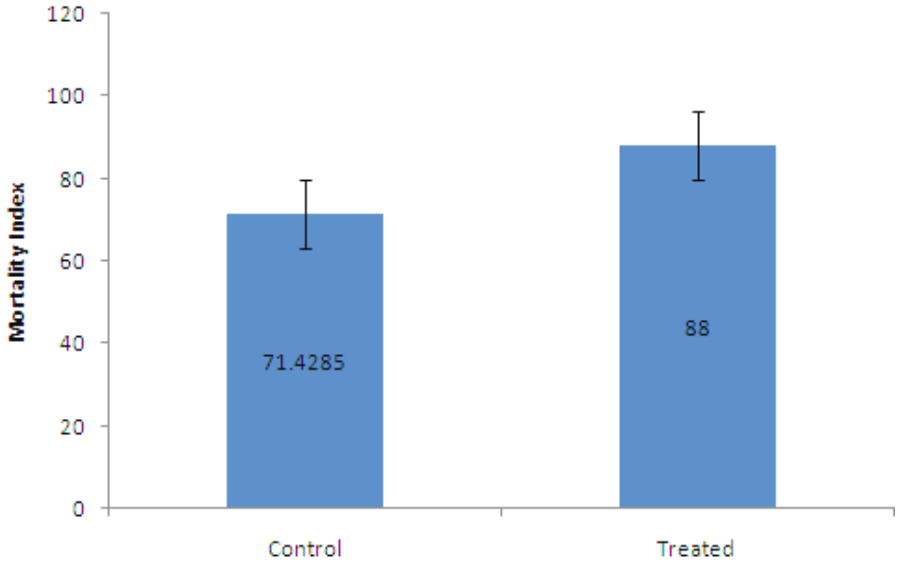

Fig. 3. Mean mortality index in normal and treated group.
Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant.

Total count of hemocytes

Mean cell count was significantly decreased in treated group (Fig. 4).

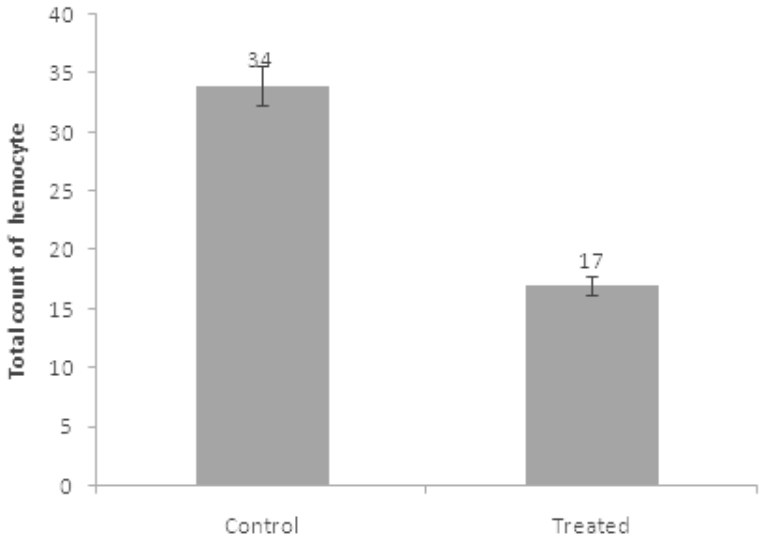

Fig. 4. Mean cell count in normal and treated group. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant.

DISCUSSION

Immunity of animals is strongly influenced by environmental conditions and factors (Girón-Pérez, 2010). Altered environmental conditions produce stress that can affect immunity by changing the functional efficiency of immune cells (Girón-Pérez, 2010; Mydlarz *et al*., 2006).

Due to high distribution worldwide, the molluscs accumulate large amount of pollutants, which is responsible for immune challenge (Bernal-Hernandez *et al*., 2010). Cellular immunity is carried in molluscs by hemocytes which can kill microbes through phagocytic reaction (Pruzzo *et al*., 2005; Ray *et al*., 2013 a, b). In the present study significant changes were observed in the cytomorphology of hemocytes when compared with the control group under light microscopy. Current scientific reports suggest apoptosis as an effective biomarker of aquatic pollution (Kiss, 2010; Ray *et al*., 2013 a,b). Cell death images in present study displayed nuclear degeneration, membrane blebbing which are the indication of apoptosis. Some percentages of hemocytes showed necrosis like features (rupture of plasma membrane). Treated cell cytoplasm in some cases, also exhibited vacuoles which resemble another type of programmed cell death (PCD), called paraptosis.

Guria *et al*. (2016) reported the toxic effect of arsenic and lead on the cytomorphology of hemocytes of grasshopper. Guria (2018) reported that lead (Pb) treatment led to formation of membrane blebs, rupture of plasma membrane, degeneration of nuclei and vacuolization in the cytoplasm of hemocytes of *Lamellidens marginalis*. The present study corroborated the earlier studies.

CONCLUSIONS

Many of the chemical pollutants are potent immunosupressors. The results reveal that arsenic affected molluscs may play a significant role in accumulating and further transferring toxic metals to higher trophic levels in the food chain. It is clear that exposure to arsenic (As) alters normal biological functions, resulting in disease or, at least, predisposition of an organism to it. Regular monitoring of arsenic levels and their associated health effects in aquatic organisms may act as bio indicator for potential impacts on food chain (Kumari *et al*., 2016). In general, the presence of toxicants in aquatic media exerts its effect at the cellular or molecular level, which results in significant changes in biochemical parameters.

By analysing the morphology, cell death of hemocytes, one can assume how much the concerned ecosystem is polluted. Hemocytes will help to establish an effective bio-indicator which may provide good systems to evaluate the toxic effects of environmental contaminants.

Acknowledgements. The author thanks Head, Post Graduate Department of Zoology and Principal, Barasat Govt. College for necessary support.

REFERENCES

ADEMA C.M., VAN W.P.W., SMINIA T., 1991, *Molluscan hemocyte-mediated cytotoxicity: the role of reacting oxygen intermediates.* Reviews in Aquatic Science, **4**: 201223.

BERNAL-HERNÁNDEZ Y.Y., MEDINA-DÍAZ I.M., ROBLEDO-MARENCO M.L., VELÁZQUEZ-FERNÁNDEZ J.B., GIRÓN-PÉREZ M.I., ORTEGA-CERVANTES L., 2010, *Acetylcholinesterase and metallothionein in oysters (Crassostrea corteziensis) from a subtropical Mexican Pacific estuary*. Ecotoxicology, **19**: 819–825.

BOGDAN C., 2001, *Nitric oxide and the immune response*. Nature Immunology, **2**: 907–916.

CHEN J.H., BAYNE C.J., 1995, *Bivalve mollusc hemocyte behaviors: characterization of hemocyte aggregation and adhesion and their inhibition in the California mussel* (*Mytilus califurnianus*). Biology Bulletin, **188**: 255–266.

CHENG T.C., 1977, *Biochemical and ultrastructural evidence for the double role of phagocytosis in molluscs: defence and nutrition.* Comparative Pathobiology, **3**: 21–30.

DUKER A.A., CARRANZA E.J.M., HALE M., 2005, *Arsenic geochemistry and health*. Environ Int, **31**(5): 631–641.

GURIA S., 2018, *Lead (Pb) induces paraptosis like cell death in hemocytes of Lamellidens sp: a preliminary study*. World Scientific News, **94** (2): 163–172.

GURIA S., PARVEEN S., GOSWAMI D.S., 2016, *Alteration of morphology, phagocytic behaviour andaggregation of insect haemocytes exposed to contaminated food with arsenic and lead*. International Journal of PharmTech Research, **9** (5): 177–186.

GIRON-PEREZ M.I., 2010, *Relationships between innate immunity in bivalve molluscs and environmental pollution.* ISJ, **7**: 149–156.

HILLYER J.F., SCHMIDT S.L., CHRISTENSEN B.M., 2003, *Hemocyte mediated phagocytosis and melanization in the mosquito Armigeres subalbatus following immune challenge by bacteria.* Cell and Tissue Research, **313**: 117–127.

HUMPHRIES J.E., YOSHINO T.P., 2003, *Cellular receptors and signal transduction in molluscan hemocytes: connections with the innate immune system of vertebrates*. Integrative and Comparative Biology, **43**: 305–312.

KISS T., 2010, *Apoptosis and its functional significance in molluscs*. Apoptosis, **15**: 313–321.

KUMARI B., KUMAR V., SINHA A.K., AHSAN J., GHOSH A. K., WANG H., DEBOECK G., 2016, *Toxicology of arsenic in fish and aquatic systems*. Environ Chem Lett, DOI 10.1007/s10311-016-0588-9

MYDLARZ L.D., JONES L.E., HARVELL D., 2006, *Innate Immunity, environmental drivers, and disease ecology of marine and freshwater invertebrates*. Annual Review of Ecology, Evolution and Systematics, **37**: 251–288.

NATH S., BOSE S., ROY B., 2012, *Effect of arsenic on haemolymph cells of short horned grasshopper*. Romanian Journal of Biology-Zoology, **57**: 163–68.

PRABHAKAR A.K., ROY S.P., 2009, *Ethno medicinal uses of some shellfishes by people of Kosi river basin in North Bihar, India*. Journal of Ethnobiology and Ethnomedicine, **3**: 1–4.

PRUZZO C., GALLO G., CANESI L., 2005, *Persistence of Vibrios in marine bivalves: the role of interactions with haemolymph components*. Environmental Microbiology, **7**: 761–722.

RAY M., BHUNIA N.S., BHUNIA A.S., RAY S., 2013a, *A comparative analyses of morphological variations, phagocytosis and generation of cytotoxic agents in flow cytometrically isolated hemocytes of Indian molluscs*. Fish Shellfish Immunology, **34**: 244–253.

RAY M., BHUNIA A.S., BHUNIA N.S., RAY S., 2013b, *Density shift, morphological damage, lysosomal fragility and apoptosis of hemocytes of Indian molluscs exposed to pyrethroid pesticides*. Fish Shellfish Immunology, **35**: 499–512.

RAY S., MUKHERJEE S., BHUNIA N. S., BHUNIA A. S., RAY M ., 2015, *Immunotoxicological threats of pollutants in aquatic invertebrates* (http://dx.doi.org/10.5772/60216).

RENWRANTZ L., SCHANKE W., HARN H., ERL H., LEIBISCH H., GERCKEN J., 1981, *Discriminative ability and the function of the immunological recognitionsystem of the snail Helix pomatia*. Journal of Comparative Physiology, **141**: 477–488.

ROSSMAN T.G., 2003, *Mechanism of arsenic carcinogenesis: an integrated approach*. Mutat Res, **533**: 37–65.

SUBBA R.N.V., DEY A., 1989, *Freshwater molluscs in aquaculture*. *In: Director(Ed.), Handbook: Freshwater Molluscs of India*. Zoological Survey, pp.: 225–282.

TSENG W.P., 1977, *Effects and dose-response relationships of skin cancer and blackfoot disease with arsenic*. Environmental Health Perspect, **19**: 109–119.

WANG C.H., JENG J.S., YIP P.K., CHEN C.L., HSU L.I., HSUEH Y.M., CHIOU H.Y., WU M.M., CHEN C.J., 2002, *Biological gradient between long-term arsenic exposure and carotid atherosclerosis*. Circulation, **105**: 1804–1809.

Received March 20, 2020

Post Graduate Department of Zoology, Barasat Govt. College, Barasat, Kolkata-700124, West Bengal, India
e-mail: srikantarna@yahoo.com; guriasrikanta@gmail.com