CIBTech Journal of Zoology ISSN: 2319–3883 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjz.htm
2018 Vol.7 (3) September-December, pp.1-5/Mahajan
Research Article

OXIDATIVE STRESS AND HISTOPATHOLOGIC BIOMARKERS OF EXPOSURE TO ARSENIC TRIOXIDE IN THE FRESHWATER MUSSELS, *LAMELLIDENS CONSOBRINUS*

P. R. Mahajan
Department of Zoology, Sardar Vallabhabhai Patel Arts and Science College, Ainpur, Tal - Raver, Dist – Jalgaon, 425509
**Author for Correspondence: prmahajan1971@gmail.com*

ABSTRACT
The toxicity of Arsenic Trioxide (As_2O_3) is well documented but it also exerts its toxic effects through multiple pathways especially by inducing a state of oxidative stress (Catalase activity) and causing damage to the vital organs. In the present study, histopathologic and oxidative damage caused by Arsenic trioxide in digestive gland of fresh water mussels, *Lamellidens consobrinus* was evaluated. LC_{50} of As_2O_3 for *Lamellidens consobrinus* was determined by probit regression analysis. Mussels were exposed to a Chronic concentration of As_2O_3 *i.e.* 0.235 ppm ($LC_{50}/_{10}$) for 12 days. Histologic studies revealed that Arsenic caused degenerative changes in digestive gland exposure of chronic concentration of arsenic caused oxidative damage in digestive gland. Catalase activity (CAT) significantly decreased in digestive gland of treated group compared to control group. From this study it is concluded that As_2O_3 caused toxic effects in mussels by changing oxidative balance and damaging the vital organs.

Keywords: *Lamellidens Consobrinus, Arsenic Trioxide, Digestive Gland, Histopathological Alterations, Catalase Activity*

INTRODUCTION

Recently, pollution of aquatic environment with heavy metal has been a worldwide problem because they are indestructible and most of them have a toxic effect on organisms (Mac Farlane and Burchette, 2000). Among environmental pollutants, metals are of particularly related due to their toxic effect and ability to bioaccumulation in aquatic ecosystems (Censi, *et al.,* 2006). Various activities by man have increased the quantity and distribution of heavy metals in the atmosphere, land and water bodies. The extent of this widespread but diffused contamination has raised concern about its hazards for plants, animals and man (Gbaruko and Friday, 2007).

Pollution of the aquatic environment by heavy metals is a major threat to human health and to aquatic organisms (Hamed *et al.,* 2015). Heavy metal pollution of water is a major environmental problem facing the modern world (Dushenkov *et. al;* 1995). Arsenic, a human carcinogen, is present in high concentration at many toxic waste sites through disposal of arsenic - containing compounds from industrial and mining practices. In addition, arsenic can contaminate ground-water and well water from natural sources (Autier *et. al*; 1998,) Certain geological formations contain high level of arsenic that can easily leach into ground water and find their way into wells and other public water supplies. This has been a major problem in certain parts of the world including area of Taiwan, South America, India and Bangala Desh. Increase of high level of arsenic in drinking water increases the risk of lung, skin, liver, bladder cancers of the population (Nagtegaal *et. al;* 1997).Many wells have arsenic concentrations in the range of 100-800 ppb (Hageoorn *et. al.*,1995)

Under normal physiological condition, animals maintain a balance between eneration and neutralization of reactive oxygen species (ROS). However when organisms are subjected to xenobiotic compounds, the rate of production of ROS, such as superoxide anion radicals (O2 •−), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH) and peroxyl radicals (ROO−) exceeds their scavenging capacity (Halliwell and Gutteridge, 2007). Enzymatic and non-enzymatic antioxidants are important defense mechanism of organisms which provide protection against environmental pro-oxidants by countering the impact of

reactive oxygen species (Tabrez and Ahmad, 2009). Therefore, antioxidant parameters and oxidative stress indices are considered potential biomarkers and are frequently used as screening tools to assess the impacts of environmental stress. Important antioxidant enzymes are catalase (CAT), superoxide dismutase (SOD), glutathione- S-transferase (GST) and glutathione peroxidase (GPx).

The aim of our study was to determine the histologic effect and compare the physiological responses of catalase (CAT) in the digestive gland of freshwater mussels,*Lamellidens consobrinus* after heavy metal stress.

MATERIALS AND METHODS

Acute study

Lamellidens consobrinus are the fresh water mussels occurring abundantly in Hartala pond at Hartala, Dist-Jalgaon of Maharashtra.For acute studies LC50 (lethal concentration with 50% mortality) was calculated for 96 hours (APHA, 2005) by probit regression analysis (Finney, 1971). Water was replaced every day and fresh toxicant was added after water renewal for 96/10 hours. Mussels were monitored at regular intervals and dead mussels were removed from aquaria.A total of 40 mussels were collected and divided into two groups of 20 each. The first group consisted by control mussels while the other group was exposed to 0.235 ppm As_2O_3 concentration of 96 hrs.upto 12 days.

Histologic studies

The experimental mussels from A and B group were dissected after 4,8,and 12 days. The digestive gland from all experimental group were removed and fixed in Bouin's fluid for 24 hours;washed and dehydrated in alcohol grades, cleared in toluene and embedded in paraffin wax (58-60^{0}c). Serial sections of 5 u thickness were cut and stained with Mallory's triple stain. The stained sections were examined under light microscope for histopathological impact of heavy metal salts. Digestive gland of mussels from both groups of control and exposed were screened and data is presented and compared.

Tissue processing

The removed wet tissue of digestive gland from all groups were homogenate in blender with M/150 phosphate buffer at 1-4^{0}c and centrifuge.stir sediment with cold phosphate buffer and allows standing in the cold with shaking occasional then repeating the extraction once or twice and using the supernatant for assay of catalase.

Biochemical analysis

Catalase activity (CAT) was measured following decrease of absorbance at 240 nm due to H_2O_2 consumption(Luck H,1974).

RESULTS AND DISCUSSION

The accumulation of heavy metals in the body of the organism leads to the formation histological lesions in the organs. chronic exposure of *Lamellidens consobrinus* to arsenic trioxide causes severe histological lesions in the digestive gland as compared to contro group of snails. Mercury Arsenic contents in *Lamellidens consobrinus* after exposure to LC $_{50/10}$ concentrations of AS_2O_3 (0.235ppm for 12 days) has been sumarised in fig.A,B,C and D.

Histological structure of digestive gland

Histological study shows two types of cells in hepatic lobules , the typical liver cells or digestive cells (columnar epithelial cells) and secretory cells. Both rest on basement membrane. . The lumen present inside the lobules. The lobules of the gland are bound together by the thin connective tissue layer as shown in fig. A . The deformity in the cytoarchitecture of digestive gland due to heavy metal stress definitely reflect in it's metabolic activity.The histological changes in the digestive gland of *Lamellidens consobrinus* exposed to acute chronic concentration, 0.235 ppm for 4 days,8days and 12 days of arsenic trioxide, show in fig.B.fig.C and fig.D. The deformity in the histologic structure of digestive gland is more in 12days exposure group of mussels as compared to another groups of B ,C and control.

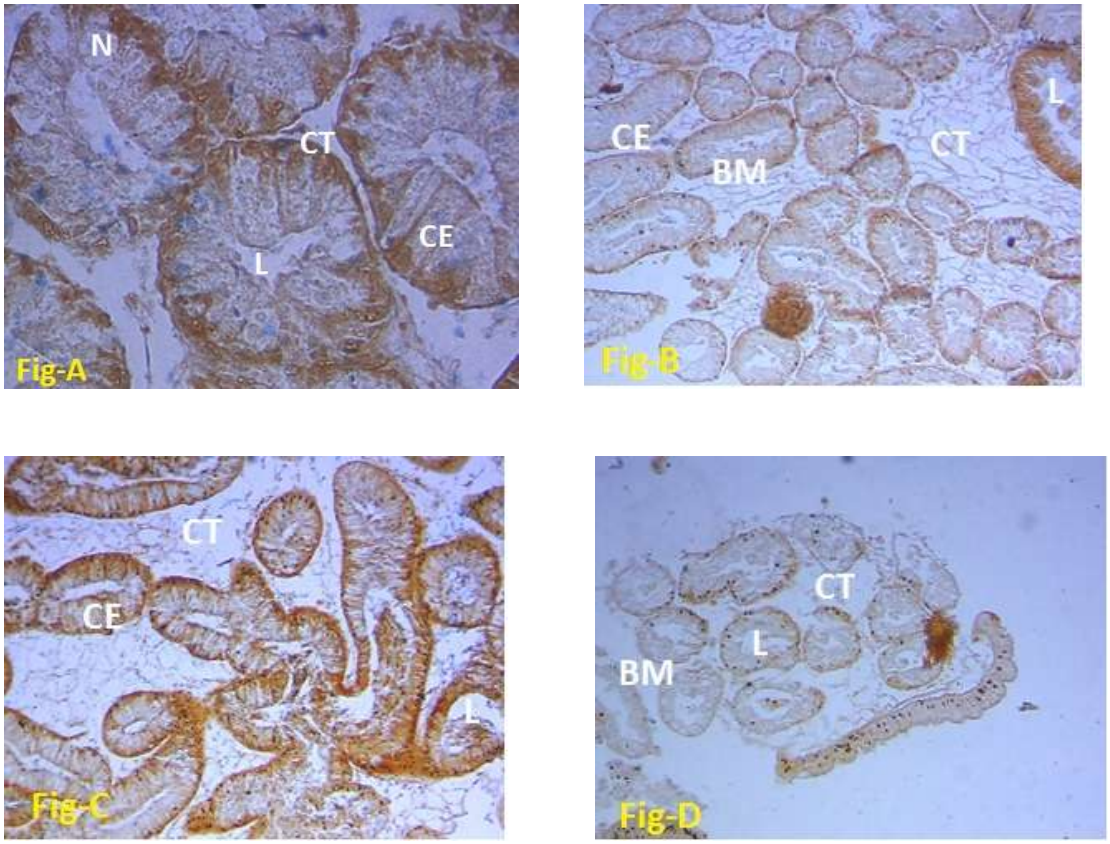

Figure: 1 A, B, C and D: Transverse sections of the digestive gland of fresh water mussel, *L.consobrinus* of the control (A), after exposure of 4 days (B), after exposure of 8 days (C) and after exposure of 12 days (D) on exposure to the chronic $LC_{50/10}$ concentrations of arsenic trioxide chloride × 150 (H & E).

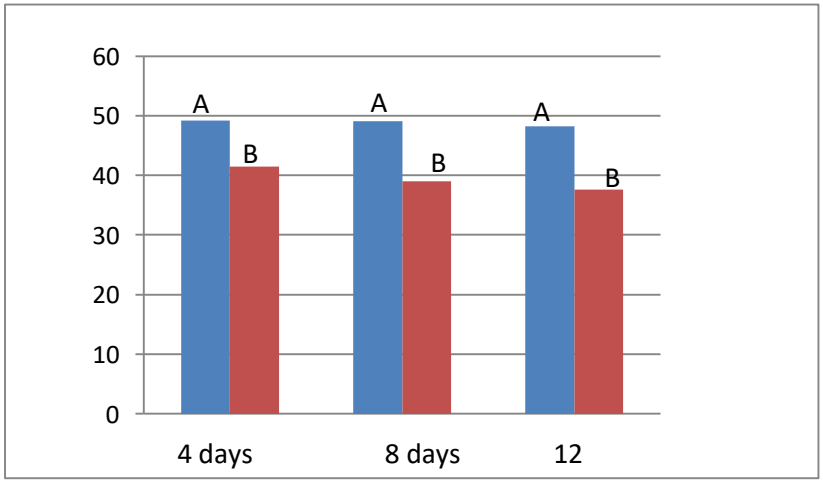

Table A: Catalase activity in digestive gland of *Lamellidens cosobrinus*

Catalase activity (CAT) showed a significant decreased with increasing exposure period of heavy metal salts, arsenic trioxide. Decrease in activity was proportional to days of exposure as well, with lowest CAT activity in digestive gland on the 12 th day. (Table A) Mean catalase activity was lowest in mussels from treatments exposed to the heavy metal concentrations as compared to control group of snailsmussels.

However, the CAT activity in digestive gland control group of mussels is 49.9 to 48.3 and in experimental group of mussels is 41.5 to 37.7. respectively.

DISCUSSION

Various anthropogenic materials are released into bodies of water which affect aquatic life especially freshwater mussels.The alterations observed in histo-architecture in the various tissues are tissue specific and time dependent.When *Channa punctatus* was exposed to mercuric chloride for a prolonged span of 30 days profound histological changes in the liver were observed which induced necrosis, vacuolation and degeneration of hepatocytes (Sastry and Gupta, 1978). The histological techniques are the promising area of research in aquatic toxicology as it gives the real picture of the effects imposed and the involvement of the xenobiotics in either disturbing or destroying the vital organs of living organisms. Many workers have reported the degenerative changes in selected tissues of the animals in response to pollution by various toxicants (Shaikh, *et al.,* 2010; Andhale, *et al.,* 2011).Victor *et al.* (1990) observed histopathological changes in the hepatopancreas of *P. hydrodromous* in response to cythion resulting in reduction in the height of tubular epithelium, enlargement of lumen, vacuolation and atrophy. The histopathological changes indicated that the animals were not able to digest and store food properly.

The present study revealed that arsenic trioxide administration induced a state of oxidative stress and changed the digestive gland histology of freshwater mussels, *Lamellidens consobrinus.* Exposure to the $LC_{50/10}$ chronic concentration of AS_2O_3 changed the normal architecture of digestive gland. Catalase activity (CAT) showed a significant decreased with increasing exposure period of heavy metal salts, arsenic trioxide.The lack of nutrientsresulted in atrophy of hepatopancreas.These degenerative changes result in the impairment of physico-metabolic processes ofmussels. Break of DNA strands in hepatopancreas of mussels exposed to resin acids was reported by Gravato et al. (2005). Siweela A.H and et.al,(2010) studied, A comparison of metal levels and antioxidant enzymes in freshwater snails, Lymnaea natalensis, exposed to sediment and water collected from Wright Dam and Lower Mguza Dam, Bulawayo, Zimbabwe and conclude that, Superoxide dismutase (SOD), diphosphotriphosphodiaphorase (DTD) and catalase (CAT) activities were significantly lower whilst malondialdehyde (MDA) levels were significantly higher in tissues of snails exposed to Lower Mguza Dam sediment and water. On the other hand, selenium-dependent glutathione peroxidase (Se-GPX) activity was significantly elevated in tissues of snails exposed to Lower Mguza Dam sediment and water. in all the organs of *Channa punctatus* exposed to deltamethrin. Chitra and Maiby (2014) studied decrease in catalase activity in the liver of fresh water fish *Oreochromis mossambicus* exposed to the sublethal concentration of BPA

The results of the present study revealed that the fresh water mussels, *Lamellidens consobrinus* undergo oxidative stress and respond by changes in catalase activity and histopathologic structure in digestive gland.

ACKNOWLEDEMENT

The authors are thankful to the Principal, Dhanaji Nan Mahavidyalaya, Faizpur for providing the laboratory facility to carryout the work.

REFERENCES

Andhale AV, Bhosale PA and Zambare SP (2011). Histopathological study of nickel induced alterations in the fresh water bivalve, *Lammellidens marginalis*, *Journal of Experimental Sciences,* **2** (4) 01-03.

Autier P, Dore JF, Cattaruzza MS, Renaed F, Luther H, Gentiloni SF, Zantedeschi E, Mazzetti MI and Andry M (1998). Sunscreen use, wearing clathes and number of nevi in 6 to 7 years old European children, *Journal of National Cancer Institue* **90** 1873 -1880.

Censi P, Spoto SE, Saiano F, Sprovieri M and Mazzola S (2006). Heavy Metals in Coastal Water System: A Cast Study from North Western Gulf of Thailand. Chemosphere **64** 1167-1176.

Chitra KC and Maiby S (2014). Oxidative stress of Bisphenol-A and its adverse effects on liver of fresh water fish *Oreochromis mossambicus*. IJSR **3**(7) 221-224.
Dushenkov V, Kumar PB, Motto AN and Raskin I (1995). Rhizofiltration : the use of plant to remover heavy metals from aqueous streams; *Environmental Science and Technology* **29** 1239-1245.
Finney DJ (1971). Probit Analysis, 3rd edition, London and New York, *Cambridge University Press*, pp. 333.
Gbaruko BC and Friday OU (2007). Bioaccumulation of heavy metals in some fauna and flora**.** *International Journal of Environmental Science and Technology* **4** 197–202.
Gravato C, Oliveira M and Santos MA (2005). Oxidative stress and genotoxic responses to resin acids in Mediterranean mussels. *Ecotoxicology and Environment Safety* **61(**2) 221-229.
Hemelraad J, Halliwell B and Gutteridge JM (2007). Free Radicals in Biology and Medicine. Fourth Edition. *Oxford University Press*, Oxford-New York **1** 851.
Hamed AS, Alim DI, Babiker EM and Saad HA (2015). Assessment of Heavy Metal pollution in the White Nile River in the Sudan, International Journal of Advance Research Vol. **3**(10) 1270-1275.
Hageoorn LU and Lippoid BC (1995). Absosrption of sunscreens and other compounds through human skin in viva. derivation of a method to product maximum mixtures, *Pharma Research* **12** 1354 - 1360.
Mac Farlane GR and Burchette M D (2000). Cellular Distribution of Copper, Lead and Zinc in the Grey Mangrove, Aviciennamarina (Forsk) Vierh, *Aquatic Botany* **68** 45-59.
Naglegaal M and Tornes TA (1997). Filtersubstanzen in wasser and fis itea UWSF - Z, *Umwelt Chem Okotox* **9** 79-86.
Shaikh FI, Ustad IR and Ansari NT (2010). Effect of heavy metal on the ovary of freshwater crab, *Barytelphusa cunicularis* [Westwood]. The Bioscan **5(** 2) 335-338.
Siwela AH, Nyathi CB and Naik YS (2010). A comparison of metal levels and antioxidant enzymes in freshwater snails, Lymnaea natalensis, exposed to sediment and water collected from Wright Dam and Lower Mguza Dam, Bulawayo, *Zimbabwe.Ecotoxicology and Environment Safety* Oct **73**(7) 1728-32.
Sastry KV and Gupta PK (1978). Effect of mercuric chloride on the digestive system of *Channa punctatus*, A histopathological study, *Environmental Research* **16** 270-278.
Tabrez S and Ahmad M (2009). Effect of wastewater intake on antioxidant and marker enzymes of tissue damage in rat tissues: implications for the use of biochemical markers. *Food Chemistry and Toxicology* **47**(10) 2465-2478.
Victor B, Jeyaraj DA and Ramakrishnan P (1990). Effect of cythion on food utilization, tissue pathology and behavior of the freshwater, *Paratelphusa hydrodromous* Herbst. Impacts of environment on animals and aquaculture eds., Manna GK and BB Jana, 265-269.