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Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

IS MERCURY POLLUTANT CAUSE DEVELOPMENTAL DEFECTS? AN *IN VITRO* CASE STUDY WITH *Chironomid* LARVA

K. Venugopal, P.P. Vignesh and K. Jayaprakash

Department of Biotechnology
Karpaga Vinayaga College of Engineering and Technology
(Affiliated to Anna University, Chennai)
Kancheepuram – 603 308, Tamilnadu, India.

Abstract

Mercury metal toxic pollution is contributed significantly by industrial waste water discharge and e-waste dumping. Its toxic nature has been studied extensively by earlier workers in animal models. However there is a lacuna on mercury and its effect on developmental biology .In this present study an attempt has been made on the in vitro reaction of larval mouth parts deformities upon mercuric chloride LC_{50} dose in *Chironomous* larva. There was considerable change in the shape and size of various mouth parts of such as Mentum, Submentum, Labrum and Mandibles. Further the application of mitotic chromosomal C-banding technique was yielded a result on the change in stained bands of 3rd left arm 41a and 42a regions with that of control chromosome. The above results are discussed with teratogenic effect of mercury pollution and *Chironomous* insect larva as biomodel studies for mercury pollution.

Key words: Mercury as teratogenic pollutant; Mercury on developmental deformities; *Chironomus* larva as biomarker; Mercury toxicity on development.

INTRODUCTION

Mercury is a heavy metal that can exit in three forms as metallic mercury (Hg), mercurous (Hg^{2+}) salts, and organic mercury form (Hg^{2++}). Metallic mercury has been found in many industrial waste discharge, e-waste dumping and spillover on earth surface. Besides emission of mercury vapour due to industrial activity also contribute atmospheric mercury vapour and fixation of solidified mercury in environmental media .The abundance of mercury present within these contaminated environmentally critical sites causes health and environmental problem to the surrounding ecosystems .Now a red alert is given by environmentalists on mercury pollutants(Sharma,2003). Chronic exposure to mercury can cause detrimental effects to human as animal health.The sediment mercury in aquatic system is converted into methyl mercury by microbial action. Methyl mercury chemical species can very easily entered into biological tissues and cellular systems... Exposure to methyl mercury can develop serious health consequences. The major route of methyl mercury is consumption of fish tissue contaminated with methyl mercury. It has been observed by number of research workers that pregnant women who consumes large amount of fish are at increased risk of exposing their fetal development to methyl mercury (Boening, 2000).

Earlier studies have exhibited that varying exposure of methyl mercury can alter the development of embryos in experimental animal. Aquatic toxicologist have known for a long

time that embryos and larval stages of lower invertebrates are often the most sensitive stages in animal's life cycle as they are subjected to the exposure of toxic pollutants. Toxicants that exert developmental defects are seriously be concerned as these pollutants cause birth defects. These pollutants at critical stages of development cause teratogenic effects, morphological structural defects and developmental deformities. The most sensitive system appears to be affected are developing skeletal system .Nevertheless stunting growth are seen in many species treated with a different teratogenic compounds. There is a lack of knowledge that mercury cause such a morphological structural defects upon exposure in environmental media. Testing developmental effects of toxicant metal aquatic pollutant may be significant to understand the nature of pollutant and to initiate abatement strategies. .Besides using insect larval forms rather than using mammalian embryos a number of advantages to bioassay the suspected compound at microcosm level (Judith and Peddrick, 1987; Clarkson, 1992; Haley, 2005).

The larval forms of *Chironomus* (*Dipterian* insect) are one such experimental organisms suggested by environmental toxicologists for the bioassay of pollutant as teratogen or mutagen. The *Chironomous* larva spend most of their life in aquatic sediment surface and they remain exposed to different pollutants. They have short life cycle. Their morphological deformities indicates the assessment of water quality. The relationship between morphological deformities occurrence of metal pollutant in their habitat are highly significant. It has been shown number of deformities in mouth parts due to various metal pollutants. Such deformities could ascertain the nature of pollutant to be studied and its effect on the developmental system (Saha and Mazumdar, 2014). Therefore in this present study *Chironomus* larvae has been attempted for the exposure to mercuric chloride to know whether mercury pollutant develop any structural deformity during the development.

MATERIALS AND METHODS

The materials of the present study were *Chironomus* larval population. They were collected from fresh water ponds located in Redhills area of Chennai city. They were cultured in laboratory as per the method outlined by Saha and Mazumdar (2014) the one day cultured larval population prior to pollutant was served as control group, they were preserved and stored in 70% ethyl alcohol. In another batch of experiments the larva were exposed with various serial dilutions of laboratory made mercuric chloride solution (0.10,0.20,0.30,0.40,0.50 and 0.60) for 24hrs.The LC_{50} toxic dose was drawn from the mortality rate by using probit - regression calculations. The critical LC_{50} dose for 24 hr was given for the experimental observations. The larval head capsules of both control as well as experimental *Chironomus* larva were removed and treated with 10% potassium hydroxide solution. Then they were washed in double distilled water and preserved in 70% alcohol. Again they were treated alcoholic phenol. The head capsules were placed ventrodorsally on micro slides. The specimens were stained with eosin and observed under digital microscope and photographed. The deformities of the various mouth parts in the experimental larval forms were scored and evaluated. The data was also statically analyzed to know the significant.

To substantiate the developmental defects upon mercury exposures, a salivary gland tissue squash mitotic chromosomal preparations were also made in this study. The salivary gland of *Chironomus* larva was dissected out. The gland tissue was squeezed and spread over on albumin pre coated micro slide. The mitotic chromosome was stained by C-band stain technique. The slides were treated with 0.2N HCl at room temperature for 20mins at 50℃ and then stained with 3% Giemsa solution at pH 6.8 for 20-30mins. The equally prepared control chromosomal bands were compared with that of experimental chromosomal bands. The details of technique is available in the technical report of Monica et al (2014).

RESULTS

The results recorded in this present investigation are given below. The serial continuous observation on the exposure of various dilutionof mercuric chloride had resulted in different mortality rates. The probit-regression calculation on the mortality data was exhibited that mercuric chloride of 0.386 mg/L exposure for 24 hr. as LC_{50}(Tables 1 & 2).Therefore for

toxicological studies on the development of *Chironomus* larva, the treatment of 0.386 mg/L. mercuric chloride was given as test dose for the experimental group.

Table.1: Probit mortality rate and derivation of LC_{50} dose of mercuric chloride

	Number	Conc	Number of larva	Observed mortality	Expected mortality	Residual	Probability
PROBIT	1	-1.000	100	24	20.284	3.716	.203
	2	-.699	100	32	34.287	-2.287	.343
	3	-.523	100	40	43.843	-3.843	.438
	4	-.398	100	46	50.886	-4.886	.509
	5	-.301	100	56	56.342	-.342	.563
	6	-.222	100	68	60.716	7.284	.607

The Figure.1 is the diagrammatic representation of the various mouth part of head capsule of *Chironomus* larva in natural habitat devoid of any known chemical pollutants (or) mutagen. In the category of experimental larva the critical toxicological dose of LC_{50} mercuric chloride was given for 24 hr. Both the control as well as experimental larval population the head capsule were stained, observed under microscope and photographed. It could be seen through the microscope there were several deformed structures in the mouth parts of larva such as Antenna, Mandible, Mentum, Submentum and labrum (Figs .2, 3 & 4). Such deformed mouthparts were not seen in control species (Fig 5). The number of deformities appeared in experimental larval head capsules were tabulated in each structure wise. Deformity in each case of mouth part was scored. The mouth part which was frequently as well as highly modified was also determined (Table 3).

Table.3Developmental deformities observed in head capsule of experimental *Chironomous* larva.

S.NO	NAME OF THE PART	NO. OF LARVA OBSERVED	NO. OF DEFORMITY	%
1	Antenna	28	16	57.14
		28	13	46
		28	11	39.28
2	Mandible	18	12	66.66
		18	15	83.33
		18	13	72.22
3	Labrum	15	8	53.33
		15	13	86.6
		15	10	66.66
4	Mantum	20	14	70
		20	17	85
		20	12	60
5	Submentum	22	16	72.72
		22	14	63.63
		22	13	59.09

From the data it was evident that there was considerable developmental deformities due to the mercuric chloride exposure when compared with the observations of corresponding control larval forms. The deserving point to be noted in this contest was the structural defects much pronounced in the cases of Mandibles, Mentum and Labrum. The percentage of deformities were 74.07%, 71.66 and 68.86%, respectively.

To know precisely about the structural modification ANOVA statistical software tool was also employed. The results was exhibited a substantial evidences to presume that said Mandible, Mentum and labrum were subjected for deformities (Fig.6). The foregoing results would unambiguously suggest that metal mercury causes developmental deformities in the *Chironomus* larva.By holding above said hypothesis, one would expect tooknow any molecular changes in the sequences of genetic materials for this developmental deformities. In this present study the same has also been observed. The comparative photomicrograph of the salivary gland mitotic auto chromosomal spread of *Chironomus*larva stained after giemsa had revealed the results on difference in the staining regions. To our surprise on comparison of healthy chromosomal stained bands of the 3rd pair left arm regions of 41a and 42a, the mercury exposed experimental larval 3rd pair chromosome had a differentially stained bands (Fig.7).

Therefore it is reasonably to suggest thatMercury metal as an environmental mutagenic pollutant and affects genetic deformity during the development of biological organisms. It is desirable to study much more clearly molecular aspects to corroborate the above concepts. Further it isalso to assume that heavy metal Mercurydevelops damage to the DNA molecules and may be describedas environmental mutagen.

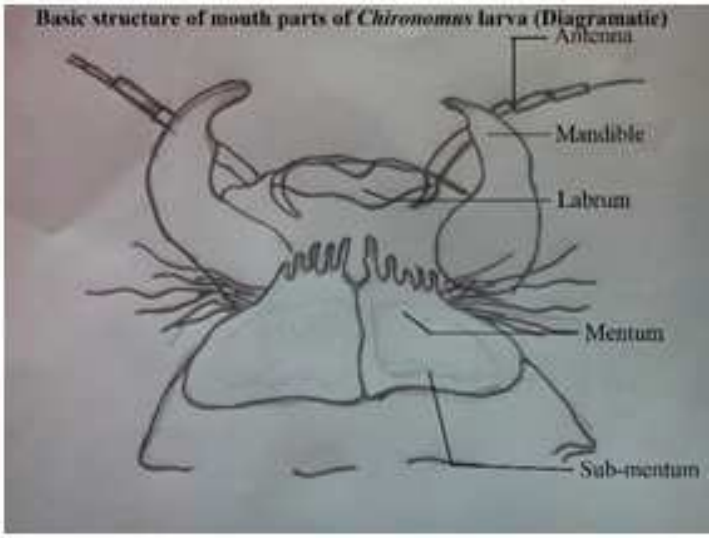

Fig.1: reference diagram of mouth parts of *chironomus* larva

Fig. 2, 3&4: Observed structural deformities in mouth parts of *Chironomus* larva exposed with Mercuric chloride

Fig. 5: Photomicrograph of head capsule of control *Chironomus* larva (unexposed)

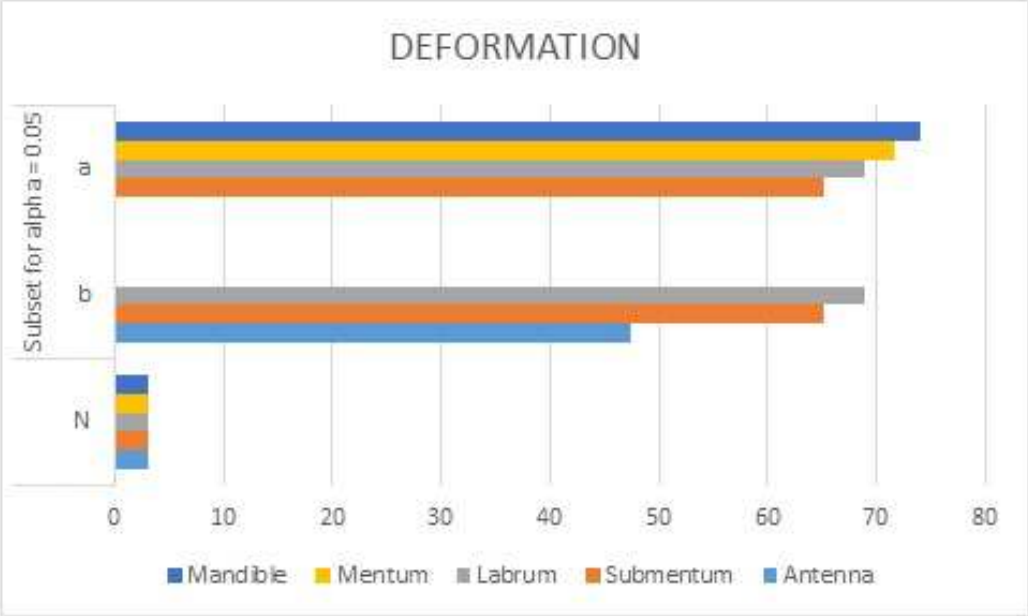

Fig. 6: Graph shows the deformation of experimental *Chironomus* larval head capsule

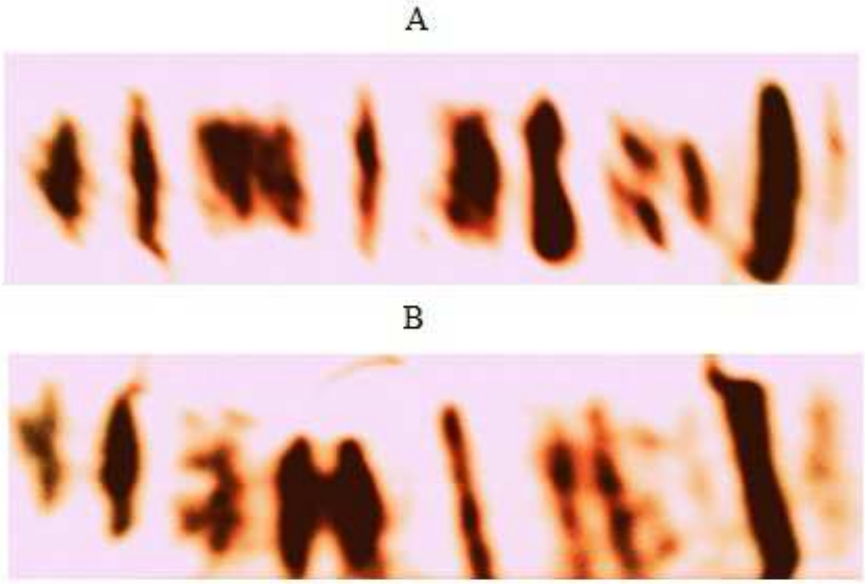

Fig. 7 Photomicrograph of 3rd pair auto chromosome left arm in the regions of 41a and 42a stained after giemsa (10x,45x). (A: control and B: experimental).

DISCUSSION

Mercury based pollutants in the environment comprise a vast and ever increasing range of compounds. The results indicated in the present study are highly significant that mercury interferes with developmental process of a biological organism. The dose of LC_{50} (0.386 $HgCl_2$/L) for 24 hours exposure had influenced the development of mouth parts. Many structural deformities could be observed upon the exposure of $HgCl_2$. Further the chromosomal

banding of mitotic chromosome also had the reflection of genotoxic nature of mercury. The above result would reasonable to suggest that mercury compound may affect the development and damage the expression of DNA. Normally the mercury binds with sulfhydryl group and interfere with enzyme action and decrease the amount of RNA, it causes molecular and structural damage to the cell membrane and reduce many enzyme mechanisms (Jayaprakash,2009). The recent studies by the school of Hamideh Abnoos et al (2013) disclosed that mercury metal exposure lead to many teratogenic morphological developmental defects in various larval stages of fruit fly Drosophila. Similarly Sadripour et al (2013) have also investigated on the occurrences of developmental defects in the pleteus larva of sea urchin particularly in cilliary arms. The present study on *Chironomous* larva also corroborates earlier observation of structural defects in invertebrate larval forms upon mercury toxicity. These findings may helpful to understand ecotoxicology of mercury pollution and serve as biomodels. Knowledge of the ability of pollutants to cause genetic toxicity in biological organism had us to consider ways in which the findings of morphologically altered organisms, namely the *Chironomus* larva help us to identify a biomarker of environmental contamination.

CONCLUSION

Industrialization in evitable to both developing and developed countries so also the environmental pollution. These nations pose a considerable risk to environmental pollution due to the use of mercury in various manufacturing process and production of electronic gadgets. There is a significant contamination has already exist in atmosphere, soil and aquatic system. If this threat is not resolved the mercury pollution problem may lead to more damage to human and animal life.

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REFERENCES:

Boening, D.W. 2000. Ecological effects, transport and fate of mercury. A general review. Chemosphere, 40: 1335-1351.

Clarkson, T.W. 1992. Mercury: Major issues in environmental health. Environ. Health Perspectives 100: 31-38.

Haley E, Boyd. 2005. Mercury Toxicity: Genetic susceptibility and synergistic effects. Medical Veritas 2: 535-542.

Hamideh. A, Masoud. F, Naser. M, Farhang. H and Razieh. J. 2013. Developmental study of mercury effects on the fruit fly (Drosophila *melanogaster*). Interdisciplinary Toxicology: 6 (1): 34-40.

Jayaprakash, K. 2009. Mercury vapor inhalation and its effect on glutothione peroxidase in goldsmiths exposed occupationally. Toxicol.Indust. Health: 25(7):463-465.

Judith S.Weis and Peddrick Weis. 1987. Pollutants as developmental Toxicants in aquatic organisms. Environmental Health Perspectives 1987, 71: 77-85.

Monica G Chirino, Luis F Rossi, Maria J bressa, Juan P.Luaces and Maria S.Merani. 2014. Dipteran chromosome: a simple method for obtaining high quality chromosomal preparations. Current Science: 107 (11): 1792-1794.

Sadripour. E, Mortazavi, M.S and Mahdavi Shahri, N. 2013. Effects of mercury on embryonic development and larval growth of the sea urchin *Echinometra mathaei* from the Persian Gulf. Iranian J. Fisheries Sci.: 12 (4): 898-907.

Saha, D and A Mazumdar. 2014. The use of head capsule deformities in *Chironomid* larva (Diptera: *Chironomidae*) To assess environmental pollution in rice fields of Hooghly District, West Bengal, India., Inter. J. Innov. Sci, Engg. & Technol.: 1 (5), 500-507.