The Effect of Mercury on the Morphology of Developing Zebrafish Embryos (*Danio rerio*)

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Abstract

The current study was carried out on zebrafish embryos at 6 h post fertilization exposing them 24 h to five concentrations (0 [negative control], 5, 10, 50, 80, 100,and 200 ppb Hg cl. Zebrafish embryos exposed to2% ethanol were positive controls (100% embryonic death). Embryos were assessed at 30, 54, 72 and 96 h post fertilization for progress in development, morphometry and morphological deformities. Embryos exposed to 5 ppb methyl mercury were healthy, showed no obvious deformities. Embryos exposed to 10, 50, 80, 100, and 200 ppb hg cl showed lighter pigmentation and smaller eyes. Embryos exposed to 80, 100, and 200 ppb hg cl showed shorter larval length.Larger yolk sac was recorded in embryos exposed to 80 ppb hg cl.Twisted tail was seen in embryos exposed to 80, 100, and 200 ppb hg cl.

Introduction

Mercury neurotoxicity causes a variety of neurological and behavioral effects in humans including central hearing loss, vestibular dysfunction,autism, mental deterioration, speech difficulty, impaired vision, weakness of the extremities and ataxia, and in some cases has proven to be fatal (Carpenter, 1994; Chang, 1977; Musiek, and Hanlon, 1999; and Takeuchi et al., 1968). Several investigations have reported hearing loss and auditory comprehension difficulties in humans exposed to mercury (Harada et al., 1977 and Myers et al.,1995)

Mercury, in both inorganic and organic forms, is an environmental toxin that impairs the function of numerous tissues, including kidney, gastrointestinal tract, heart and central nervous system (Ganote et al., 1974; Bansal et al., 1985; Aschner et al., 1991; Fitzgerald and Clarkson, 1991;

Pamphlett and Waley, 1996; Diamond and Zalups, 1998).In humans, acute exposure to inorganic mercuric chloride (HgCl2) causes epithelial dysfunction, primarily in renal and gastrointestinaltissues (Ganote et al., 1974; Lin and Lim, 1993; Diamond and Zalups,1998).In fish, gill epithelia are impaired by acute exposure to HgCl2 (Lock et al., 1981; Daoust et al., 1984; Allen, 1994; Oliveira Ribeiro et al., 2000; Gupta and Dua, 2002).In the shark rectal gland, a marine organ highly specialized for NaCl secretion (Forrest, 1996; Silva et al., 1993), inorganic salts impair chloride secretion, whereas organic mercurial compounds have no effect (Silva et al., 1992).

Mercury is a heavy metal that affects the activity of several enzymes, ion channels or receptors (Prabhu and Salama, 1990; Boraso and Williams, 1994; Hulme et al., 1990; Chiamvimonvat et al., 1995) by combining with SH groups (Halbach, 1990) that are necessary for their normal function. Mercury causes depression in papillary muscles (Halbach, 1989; Oliveira et al., 1994a; Vassalloet al., 1999) and ventricular pressure in vivo and in vitro (Su and Chen, 1979; Rhee and Choi, 1989; Massaroni et al., 1992, 1995).

Hg clinhibits sarcoplasmic reticulum activity (Ahammad-Sahib et al., 1988; Hechtenberg and Beyersmann, 1991; Temma et al., 1978) leading to a calcium overload. Mercury triggers calcium released from sarcoplasmic reticulum (Prabhu and Salama, 1990). Also it reduces the activity of the myosin ATPase (Vassallo et al., 1999). Inorganic mercury is neurotoxic (Albrecht, 1996)

Organic and inorganic mercury disrupt ion channel functions so disturb cellular homeostatsis and synaptic transmissio(Sirosis, 1996). Mercury, both the organic and inorganic forms, inhibits protein synthesis in neural tissue (Verity et al., 1977; Cheung & Verity, 1983); however, in other reports Hg stimulated protein synthesis.Human monocytes treated withmercury also exhibited changes in lipid organization within theirplasma membranes. (InSug et al.,1997).The hemolyticaction ofmercuric ions on the erythrocytemembrane was studied. (Zolla et al., 1997)

Materials and Methods

Zebrafish Embryos

Adult fish were raised and kept under standard laboratory conditions at 28.5◦C (Westerfield, 2000) in the Department of Biology at Texas A&M University. Male and female adult zebrafish were paired in the evening and fertilized embryos were obtained at 10 to 11 AM the following morning. Medium, consisting of ultrapure water containing low concentrations of specific ions and adjusted to pH 7.2,

was used to maintain the developing zebrafish embryos and was freshly prepared for each experiment according to Westerfield (2000). All zebrafish embryos were staged and fixed at specific hours post fertilization (hpf) as described by Kimmel et al. (1995). Both adult and embryonic zebrafish embryos were maintained according to protocols that were carried out in accordance with the *National Institutesof Health Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, revised 1996).

Mercuric chloride Preparation

Mercuric chloride was conducted in 24-well flat bottom plates with low evaporation lids (BD Biosciences, San Jose, CA, USA). The total volume of zebrafish medium in each well was 2 ml. Four wells on each plate were used for negative controls and contained only zebrafish medium. Four wells on each plate were usedas positive controls and contained zebrafish medium with 2% ethanol (Mindel, 2000). The remaining 16 wells were divided into four wells, each containing 2 ml of zebrafish embryo medium and one of four different doses of mercuric chloride (5, 10, 50, 80, 100, 200, 500, 1500 ppb hg cl). Two - fourzebrafish embryos 6 hpfwere added to each well, the 24-well plates were prepared in duplicate and a total of 96 zebrafish embryos were tested. The 24-well plateswere covered with low evaporation lids and incubated for 96 h at 28.5◦C (Thelco Laboratory Incubator; Cole-Palmer Instrument, Vernon Hills, IL, USA).

Morphological Assessment

The effects of exposure to different concentrations of mercuric cloride on zebrafish embryo morphology were assessed at 30, 48, 72, and 96 hpf, using a SZ-40 binocular microscope (Olympus, Center Valley, PA, USA). Images of embryos at different hpf were captured using an Eclipse E400 microscope equipped with a 2× objective, a DXM1200 digital camera, and ACTI imaging software (Nikon Instruments, Melville, NY, USA). Zebrafish embryos were examined at room temperature (25◦C) to monitor the developmental stage, mortality, hatching, response to touch, and the presence of any deformities. Dead embryos were removed at each monitoring time point and all surviving embryos were photographed.We used Image J software for measurement of the embryos).

Statistical Analysis

Each treatment group consisted of eight individual values. One-way analysis of variance (ANOVA) was performed to assess differences among concentrations of mercuric chloride expressed as mean ±

standard error of the mean (*SEM*). Significance was set at $p \leq .05$ and treatment mean comparisons were made using multiple comparisons Duncan's difference procedure (Duncan 1955).

Results

At 96 hpf, Noneof the larvae exposed to 2% ethanol and1500 ppb Hg cl were alive. We first performed morphological assessments at 96hpf, zebrafish embryos not exposed to mercuric chloride or ethanol (i.e., negative-control embryos) exhibited normal age-specific developmental features, including rapid lengthening of the body; straightening of the body axis from its curvature around the yolk sac, blood vascular circulation is established with a beating heart, initiation of epidermal pigmentation. No deformities were observed in the head, eyes, yolk sac, trunk, or tail of control zebrafish embryos at 96hpf. Embryos exposed to 10 and 50 ppb hg cl also displayed only lower pigmentation as shown in Fig(4),plus tail twisting in about 75% of the embryos exposed to 80 and 100 ppb Hg cl (Fig4). So these results declared the developmental toxicity of the Hg cl while the same dose of Hg cl killed all embryos at 72 hpf.

We measured the length of the zebrafish larvae at 96 hpf. We found the embryos exposed to 80 and 100 ppb Hg cl are shorter than the control and that exposed to 5, 10 and 50 ppb Hg cl (Fig1). We also measured the yolk sac area of the larvae at 96 hpf, we found the yolk sac of the control and embryos exposed to 80 ppb Hg cl are larger than that of the control and that of the embryos exposed to 5 and 50 ppb Hg cl (Fig 2).Regarding the eye area of the larvae at 96 hpf, we found the larvae of the embryos exposed to 50, 80, and 100 ppb Hg clare smaller than that of the control and that of the embryos exposed to 5 and 10 ppb Hg cl (Fig 3).

In case of embryos exposed to 200 ppb hg cl, the massive degeneration of the whole body of the larvae is quit clear (Fig 4). Negative-control zebrafish embryos and that exposed to 5, 10, and 50 ppb Hg cl revealed significant development in all primary organ systems, but especially in the head and pectoral fins, as shown in Fig (4).

Starting at 24 hpf, zebrafish embryos begin to show motor responses to touch as well as spontaneous movement of the body; the tail coiled rapidly followed by a slower relaxation phase. Negative-control zebrafish embryos and embryos exposed 5, 10, 50 ppb Hg cl exhibited normal spontaneous movements and rapidly responded to touch.

Discussion

The increasing public concern over environment pollution from industrial and agriculture sources has encouraged scientists to explore the effect of these polutants. The developing zebrafish embryos are becoming an important vertebrate animal model to test the potential toxicants and to study mechanisms of toxicity. In this study, we observed that exposure of developing zebrafish embryos to low concentrations of hg cl adversely affect the development, whereas higher concentrations were lethal. Hg cl is present in the industrial wastes. The observed toxic effects of hg cl on zebrafish embryos at concentrations 5, 10, 50, 80, and 100 ppb might be due to that Hg cl acts selectively on microtubules (Sager , 1988) or due to inhibition of protein synthesis in the neural tissue (Verity et al., 1977; Cheung & Verity, 1983).

Our results of the larval length indicates that the zebrafish embryos exposed to 80 and 100 ppb mercuric chloride showed decreased embryonic development with respect to hatching time compared to non-exposed negative-control and that exposed to 5, 10 and 50 ppb Hg cl embryos. While the eye area results proved that the decrease of the embryonic development begin at 100 ppb Hg cl and this may be due to inhibition of protein synthesis in neural tissue (Verity et al., 1977; Cheung & Verity, 1983). However, the results of the larval length indicate that the harmful effect of the Hg cl on the zebrafish embryos begins at 80 ppb concentration. This study is the first record to show these results in zebrafish. The toxic effect of mercury might be due to killing and decreasing the metabolism of the cells, these results are supported by our results of the yolk sac area which was larger in the larvae of the embryos exposed to 80 ppb than that of embryos exposed to 5, 10 and 50 ppb Hg cl. Membrane fluidity also controls the enzyme activities associated with biological membranes and has an important role in ion transport (Ma and Xiao, 1998).

References

Ahammad-Sahib, K.I., Moorthy, K.S., Cameron, J.A., Desaiah, D., (1988): Isoproterenol potentiation of methyl mercury effects in vivo on cardiac ATPases and 3H-dopamine uptake. Bull. Environ. Contam. Toxicol. 40: 249–254.

Albrecht, J. (1996): a potential mediator of inorganic mercury neurotoxicity, Metab. Brain Dis. 11 (2): 175–184.

Allen P. (1994): Distribution of mercury in the soft tissues of the blue tilapia Oreochromisaureus (Steinda-

chner) after acute exposure to mercury (II) chloride. Bull Environ ContamToxicol 53: 675–683.

Aschner M., Chen R., Kimelberg H.K. (1991): Effects of mercury and lead on rubidium uptake and efflux in cultured rat astrocytes. Brain Res Bull 26: 639–642.

Bansal S.K., Murthy R.C., Chandra S.V. (1985): The effects of some divalent metals on cardiac and branchial Ca21-ATPase in a freshwater fish Saccobranchusfossilis. Ecotoxicol Environ Saf 9: 373–377.

Boraso, A. and Williams, A.J., (1994): Modification of the gating of the cardiac sarcoplasmic reticulum Ca2q- release channel by H2O2 and dithiothreitol. Am. J. Physiol. 267: H1010–H1016.

Carpenter, D.O.(1994): The public health significance of metal neurotoxicity, Cell. Mol. Neurobiol. 14 (6): 591–597.

Chang, L.W., (1977): Neurotoxic effects of mercury— a review, Environ. Res. 14 : 329– 373.

Cheung, M. K., and Verity, M. A. (1983): Experimental methyl mercury neurotoxicity: Similar in vivo and in vitro perturbation of brain cell-free protein synthesis. *Exp. Mol. Pathol.* 38: 230–242.

Chiamvimonvat, N., O'Rourke, B., Kamp, T.J., Kallen, R.G., Hofmann, F., Flockerzi, V., et al., (1995): Functional consequences of sulfhydryl modification in the pore-forming subunits of cardiovascular Ca2q and aqchannels. Circ. Res. 76: 325–334.

Daoust P.Y., Wobeser G., Newstead J.D. (1984): Acute pathological effects of inorganic mercury and copper in gills of rainbow trout. Vet Pathol 21: 93–101.

Diamond G.L., Zalups R.K. (1998): Understanding renal toxicity of heavy metals. Toxicol Pathol 26: 92–103.

Duncan, D. B. (1955): Multiple ranges and multiple F test. Biometrics,11: 1–42.

Fitzgerald W.F. and Clarkson T.W. (1991): Mercury and monomethylmercury: present and future concerns. Environ Health Perspect 96:159–166.

Forrest J.N. Jr. (1996): Cellular and molecular biology of chloride secretion in the shark rectal gland: regulation by adenosine receptors. Kidney Int., 49:1557–1562.

Ganote C.E., Reimer K.A., Jennings R.B. (1974): Acute mercuric chloride nephrotoxicity. An electron microscopic and metabolic study.Lab Invest 31: 633–647.

Gupta N. and Dua A. (2002): Mercury induced architectural alterations in the gill surface of a fresh water fish, Channapunctatus. J.Environ Biol., 23: 383–386.

Halbach, S., (1989): Sulfhydryl-induced restoration of myocardial contractility after alteration by mercury. Arch. Toxicol. 13 (Suppl.): 349–352.

Halbach, S., (1990): Mercury compounds: lipophilicity and toxic effects on isolated myocardial tissue. Arch. Toxicol. 64: 31–5319.

Harada, M., T. Tsukayama, S. Tatetsu, **(1977):** Psychiatric aspects of Minamatadisease, SeishinShinkeigaku Zasshi, 79 (8): 393– 413.

Hechtenberg, S. and Beyersmann, D., (1991): Inhibition of sarcoplasmic reticulum Ca2q-ATPase activity by cadmium, lead and mercury. Enzyme 45, 109–115.

Hulme, E.C., Birdsall, N.J.M., Buckley, N.J., (1990): Muscarinic receptor subtypes. Annu. Rev. Pharmacol. Toxicol., 30: 633–673.

InSug, O., Datar, S., Koch, C.J., Shapiro, I.M. and Shenker, **B.J. (1997):** Mercuriccompounds inhibit human monocyte function by inducing apoptosis: evidence for formation of reactive oxygen species, development of mitochondrial membrane permeability transition and loss of reductive reserve. Toxicology 124: 211-224.

Kimmel C.B., Ballard W.W, Kimmel S.R., Ullmann B., Schilling T.F. (1995): Stages of embryonic development of the zebrafish. Dev. Dyn., 203: 253–310.

Lin J.L. and Lim P.S. (1993): Massive oral ingestion of elemental mercury. J ToxicolClinToxicol 31: 487–492.

Lock R.A., Cruijsen P.M., van Overbeeke A.P. (1981): Effects of mercuric chloride and methylmercuric chloride on the osmoregulatory function of the gills in rainbow trout, *Salmogairdneri,* Richardson. Comp Biochem Physiol—C: Comp Pharmacol 68C: 151–159.

Ma L. and Xiao P.G. (1998): Effects of Panaxnotoginsengsaponins on platelet aggregation in rats with middle cerebral artery occlusion or in vitro and on lipid fluidity of platelet membrane. Phytother. Res., 12: 138–140.

Massaroni, L., Oliveira, E.M., Stefanon, I., Vassallo, D.V., (1992): Effects of mercury on the mechanical and electrical activity of the Langendorff-perfused rat heart. Braz. J. Med. Biol. Res. 25, 861–864.

Massaroni, L., Rossoni, L.V., Amaral, S.M.C., Stefanon, I., Oliveira, E.M., Vassallo, D.V.,(1995): Haemodynamic and electrophysiological acute toxic effects of mercury in anaesthetized rats and in Langendorff perfused rat hearts. Pharmacol. Res. 32, 27–36.

Mindel, J. (2000): The effects of ethanol on zebrafish development. DB Lab website. http://www.swarthmore.edu/NatSci/sgilber1/DB lab/ Fish/Ethanol 1.html.

Musiek, F.E. and Hanlon, D.P. (1999): Neuroaudiological effects in a case of fatal dimethylmercury poisoning, Ear Hear.20 (3): 271– 275.

Myers, G.J., P.W. Davidson, C. Cox, C.F. Shamlaye, M.A. Tanner, O. Choisy, J. Sloane-Reeves, D. Marsh, E. Cernichiari, A. Cho (1995): I Neurodevelopmental outcomes of Seychellois children sixty-six months after in utero exposure to methylmercury from a maternal fish diet: pilot study, Neurotoxicology 16 (40): 639–652.

Oliveira, E.M., Vassallo, D.V., Sarkis, J.J.F., Mill, J.G. (1994): Mercury effects on the contractile activity of isolated heart muscle. Toxicol. Appl. Pharmacol. 128: 86–91.

Oliveira Ribeiro C.A, Pelletier E., Pfeiffer W.C., Rouleau C. (2000): Comparative uptake, bioaccumulation, and gill damages of inorganic mercury in tropical and nordic freshwater fish. Environ Res 83: 286–292.

Pamphlett R. and Waley P. (1996): Motor neuron uptake of low dose inorganic mercury. J NeurolSci 135: 63–67.

Prabhu, S. D. and Salama, G. (1990): The heavy metal ions Agq and Hg2q trigger calcium release from cardiac sarcoplasmic reticulum. Arch. Biochem. Biophys. 277: 47–55.

Rhee, H.M. and Choi, B.H., (1989): Hemodynamic and electrophysiological effects of mercury in intact anesthetized rabbits and in isolated perfused hearts. Exp. Mol. Pathol. 50: 281–290.

Sager P.R. (1988): Selectivity of methyl mercury effects on cytosk eleton and mitotic progression in cultured cells. Toxicol. Appl. Pharmacol. 94 (3): 473–486.

Silva P., Epstein F.H., Solomon R.J. (1992): The effect of mercury on chloride secretion in the shark (Squalusacanthias) rectal gland. Comp Biochem Physiol C 103: 569–575.

Silva P., Epstein F.H., Karnaky K.J. Jr., Reichlin S., Forrest J.N. Jr. (1993): Neuropeptide Y inhibits chloride secretion in the shark rectal gland. Am J Physiol 265 (Part 2): R439–446.

Sirosis, J.E. and Atchison, W. D. (1996): Effect of mercurial on ligand- and voltage-gated ion channels: a review, Neurotoxicology 17: 63– 84.

Su, J.Y.,and Chen, W.J., (1979): The effects of methylmercury on isolated cardiac tissues. Am. J. Pathol. 95: 753–764.

Takeuchi, T., H. Matsumoto, M. Sasaki, T. Kambara, Y. Shiraishi, Y. Hirata,M. Nobuhiro, H. Ito,(1968) : Pathology of Minamata disease, Kumamoto Med. J. 34: 521–524.

Temma, K., Akera, T., Ku, D.D., Brody, T.M., (1978): Sodium pump inhibition by sulfhydryl inhibitors and myocardial contractility. Naunyn-Schmiedeberg's Arch. Pharmacol. 302: 63–71.

Vassallo, D.V., Moreira, C.M., Oliveira, E.M., Bertollo, D.M., Veloso, T.C. (1999): Effects of mercury on the isolated heart muscle are prevented by DTT and cysteine. Toxicol. Appl. Pharmacol. 156: 113–118.

Verity, M. A., Brown, W. J. Cheung, M. and Czer, G. (1977): Methyl mercury inhibition of synaptosome and brain slice protein synthesis: in vivo and in vitro studies. J. Neurochem. 29: 673–679.

Zolla, L., Lupidi, G., Bellelli, A. and Amiconi, G. (1977): Effects of mercuric ions on human erythrocytes. Relationhip between hypotonic swelling and cell aggregation. Biochim. Biophys. Acta 1328: 273-280.

Westerfield, M. (2000): The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*), 4th ed. Eugene, OR: University of Oregon Press.

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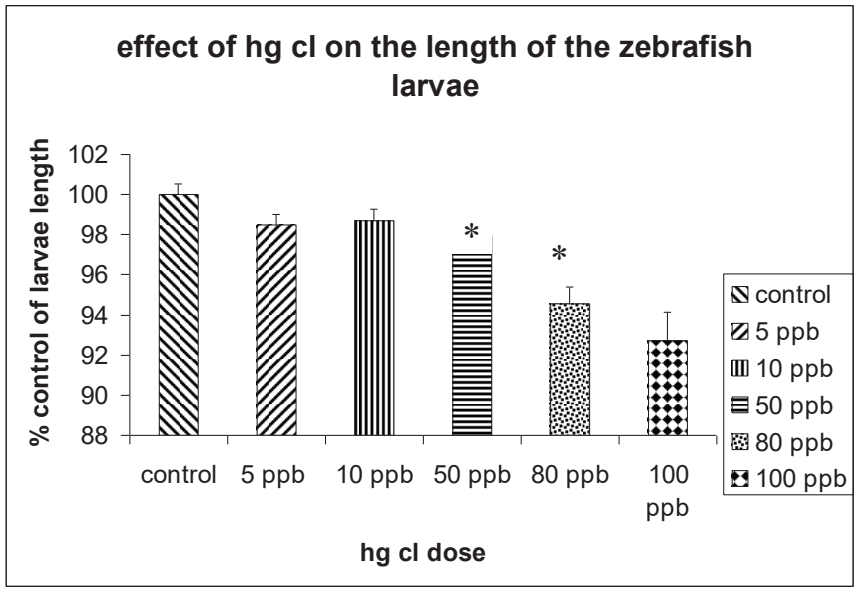

Fig (1): A histogram showing the effect of Hg cl on the larval length of zebrafish.

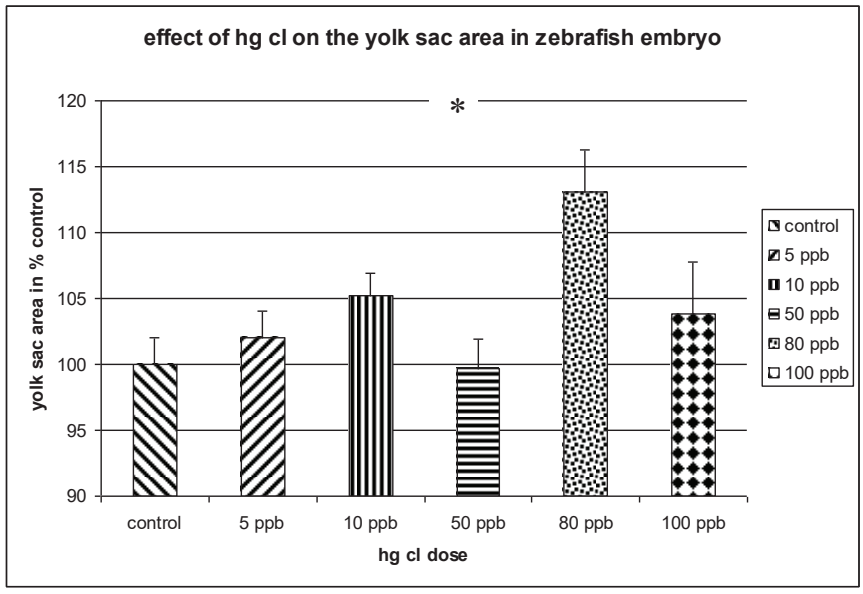

Fig (2): A histogram showing the effect of Hg cl on the larval yolk sac area ofzebrafish.

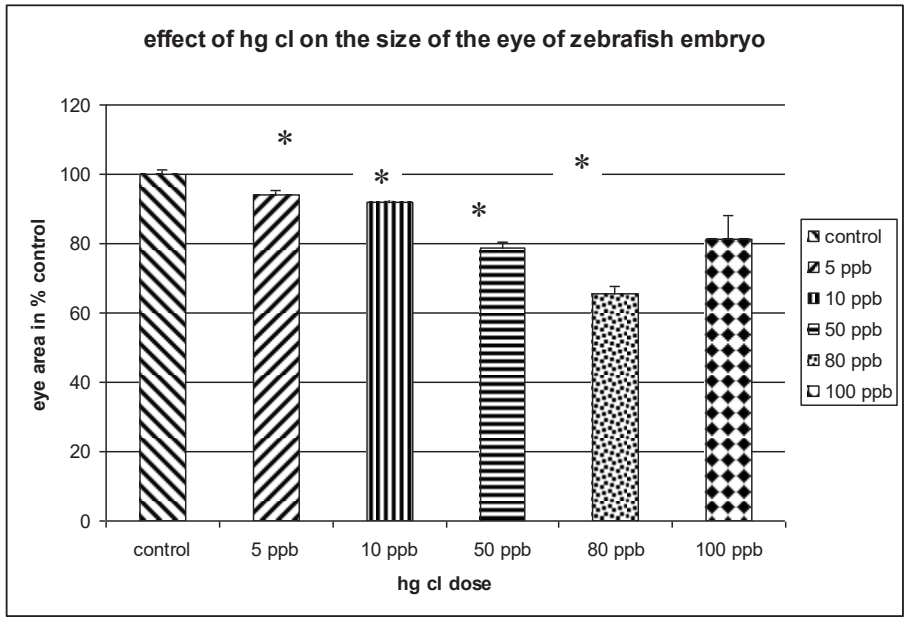

Fig (3): A histogram showing the effect of Hg cl on the eye area of zebrafish larva.

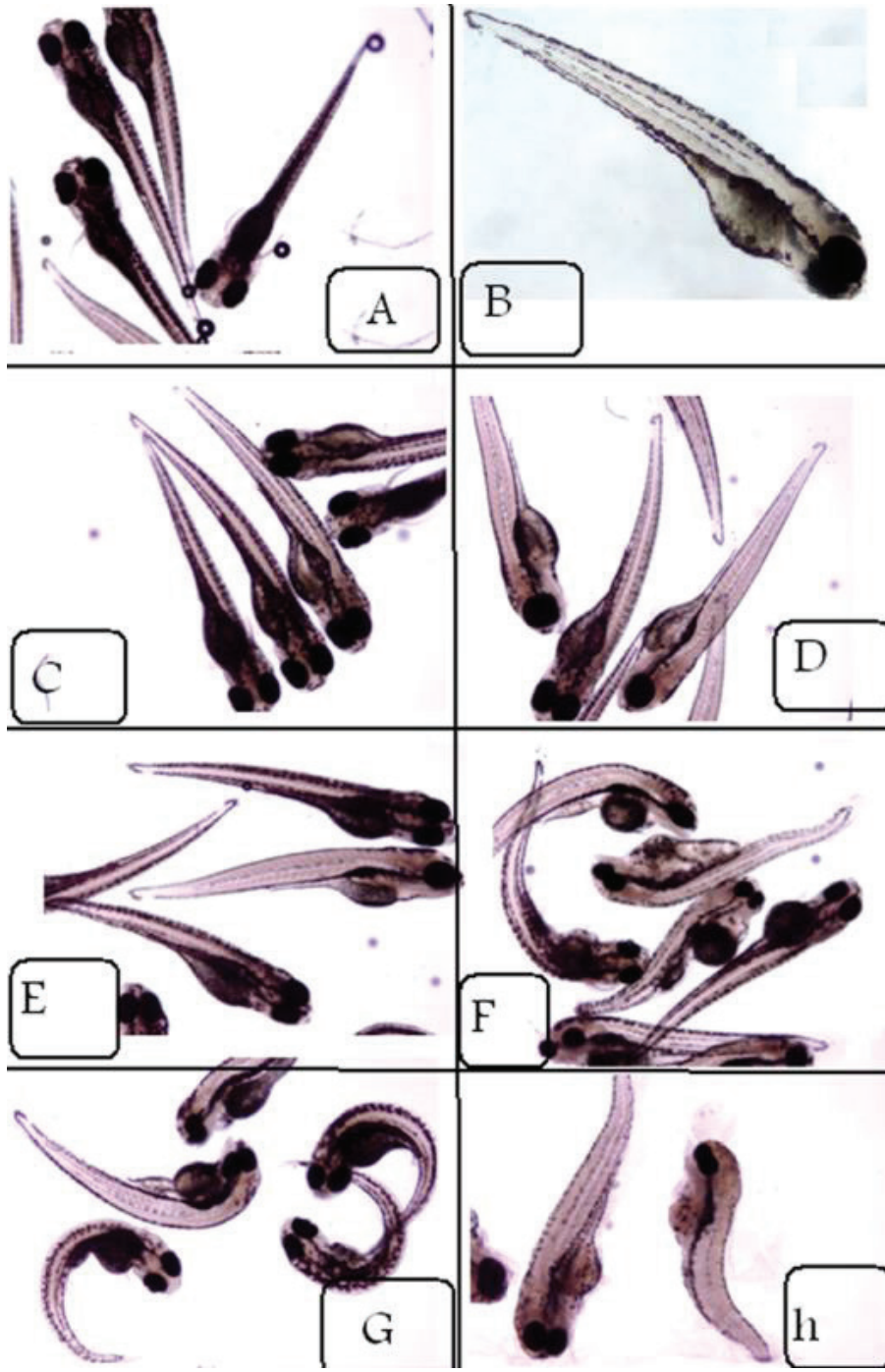

Fig (4): Aa photograph of zeprafish embryo control (A), hg cl treated at doses 5 ppb (B), 10 ppb (C), 50 ppb (D), 80 ppb (E), 100ppb (F) and 200 ppb (G &h).