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A Study and Interpretation of the Blood Cell Morphology on Related Review of Literature

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Contamination of aquatic ecosystems with different kind of pollutants has been a matter of concern over the past several decades (Myers et al, 1987; Baumann et al, 1995; Viarengo et al, 2007). Industrialization has spoiled the aquatic environment by dumping more and more concentrations of several toxic metals. The heavy metals like lead and mercury cause severe toxicity as there are established cases like Minamata episode in Japan. The mercurial toxicity and Minamata disease exhibited significant neurotoxicity similar to “Hunter Russell syndrome”. Much of research on metal toxicity relies on studies made in different animal species. Many heavy metals are poisonous above their threshold levels. The heavy metals usually enter the body through respiration, ingestion and skin. The speedy urbanization and industrialization has led to increased disposal of pollutants like heavy metals, radio nuclides, and various types of organic and inorganic substances into the environment. Thus, the industrial wastes are the main source of metal pollution for aquatic organisms. It has been cited that the heavy metals constitute the major pollutants in the environment. The heavy metals are important pollutants for fish, because these are not eliminated from aquatic system by natural methods such as organic pollutants and are enriched in mineral organic substances. The metal contaminants are mixed in the aquatic system through smelting process, effluents, sewage and leaching of garbage which cause severe harm to the aquatic system. Tannery industry has also added pollutants to the aquatic environment. The tannery waste waters continue to cause hazardous effect on the aquatic organism as they also induce endocrine disruption. Due to this, the agricultural lands are also degraded. Uncontrolled release of effluents has increased the health risks of different organisms. Interpretation of the blood cell morphology, erythrocytes particularly, became an important bio-indicator of pollution. Over recent years, changes of erythrocyte nuclei have been increasingly used to evaluate genotoxic effects of different compounds such as polycyclic aromatic hydrocarbons, naphthalene and β-naphthoflavone; (Gravato and Santos, 2002) and Heavy metals i.e. cadmium, mercury (Ayllon and Garcia-Vazquez, 2000) and textile mill effluent (Cavas and Ergene-Gozukara, 2003). Investigations are mainly conducted on fish since they have nucleated erythrocytes and are suitable for testing the pollution of aquatic ecosystems. Carrasco et al (1990) categorized these nuclear abnormalities into four groups (blebbed, lobed, notched and vacuolated nuclei). Although they did not find a consistent correlation between any variation of nuclear morphology and measured level of chemical contamination, many researches later included these abnormalities in the assessment of genotoxicity as a complementary test to micronucleus assay (Ayllon and Garcia-Vazquez, 2000; Barsiene et al, 2006; Bolognesi et al, 2006; Cavas and Ergene-Gozukara, 2005; Fenech, 2000; Gravato and Santos, 2002; Pachecco and Santos, 2002). In fish both micronuclei and erythrocyte nuclear abnormalities also appear spontaneously and their frequency can be seasonally dependent (Bolognesi et al, 2006; Jiraungkoorskul et al, 2008 ;). In other non-mammalian species the occurrence of nuclear abnormalities were recorded in birds (Gomez-meza et al, 2006), amphibian (Barni et al, 2007; Marques et al, 2009) and some reptilian species (Zuniga-Gonzalez et al, 2000). Moreover, similar anomalies were observed in fibroblasts, osteosarcoma cell line exposed to irradiation and different tumor types (mesenchymal as well as epithelial) regardless the grade of malignancy (Gisselsson et al; 2001), folic acid deficient human lymphocytes (Fenech and Crott, 2002) and laminopathies (Jacob and Garg, 2006; Mattout et al, 2006; Vigouroux et al, 2001).

The micronuclei test is employed both for laboratory assays of genotoxicity of many compounds and for in situ surveys to assess the risk of mutagen- polluted environments. The in-situ detection of environmental contaminants using fish micronuclei assay has been carried out in marine coast waters, rivers and lakes by several authors. However, the clastogenic effects of pollutants can be measured in different target tissues such as erythrocytes, gills, kidneys and liver etc. (Williams and Metcalfe 1992; Hayashi et al, 1998), but the erythrocyte micronuclei test has been used with different fish species to monitor aquatic pollution displaying mutagenic features (De Flora et al, 1993; Kligerman, 1982) demonstrated that fish inhabiting polluted water have greater frequencies of micronuclei then those of clean water fishes.

The development of in-vivo genotoxicity assay using fish as model (Powers, 1989) was enhanced by their easy handling in the laboratory. Micronucleus test detects both clastogenic and aneugenic effects (Mersch and Beauvais, 1997). Therefore, it allows the detection of genotoxicity of wide range of compounds. In addition, results of micronucleus test are rapidly obtained if carried out on haematopoetic tissues. In surface waters, fish are important links in the food chains, and may often accumulate large amounts of certain metals above the normal valves in the aquatic environment (Ambedkar and Muniyan, 2011). As a result, fish are often used as indicators of heavy metal contamination because they occupy high trophic levels and are an important source of food (Blasco et al, 1998; Agah et al, 2009). Fish are widely consumed in many parts of the world and polluted fish may harm human health (Zhang et al, 2007). Studies have revealed that fish assimilate these heavy metals through ingestion of suspended particulates, food materials or through constant ion exchange processes of dissolved metals across the lipophilic membranes such as the gill, or through adsorption of dissolved metals onto tissue and membrane surface (Oguzie and Okosodo, 2008).

The MN test in fish erythrocytes was widely validated for laboratory testing with different species after exposure to a large number of genotoxic agents. A dose response increase in MN frequency was observed after exposure to x-rays (Gustavino et al; 2001). Positive responses were also observed after exposure to a large number of carcinogens, such as aflatoxins, benzidine, EMS, methylcholanthrene, chlorinated hydrocarbons (Al-Sabti,1995), cyclophosphamide (Ayllon et al, 2000), to the most common carcinogenic pollutants, such as PAHs (Al-Sabti,1995; Pachecco and santos, 2002), pesticides (Grisolia et al, 2001; Ali et al, 2008), heavy metals (Al-Sabti,1995; Bolognesi et al,2006) and ubiquitous environmental contaminants, e.g. bisphenol A, tetrabromodiphenyl ether (Bolognesi et al, 2006) and domoic acid (Cavas and Konen, 2007).The analysis of the baseline MN frequencies reported by different authors (Ueda, et al,1992; Anitha, et al, 2000; Cavas and Konen, 2007; Al-Sabti,2000; Al-Sabti et al,1994) show a large interspecies variability, ranging from 0 to 13 per 1000 cells, although the large majority of papers report data ranging from 0 to 1. This variability can be related to an interspecies difference in metabolic competency and DNA repair mechanisms as well as cell proliferation in the target organ affecting the MN expression. A significant difference (up to one two orders of magnitude) in the MN baseline frequency was recorded in the same species by different authors, e.g. Cyprinus carpio or Oncorhynchus mykiss, that can be correlated to different biotic factors such as age, sex, genetic make –up, conditions of treatment or different scoring criteria.

Different studies have documented the peripheral erythrocytes have a high incidence of micronuclei after exposure to different toxicants both under field and laboratory conditions. During micronucleus study, intraspecific factors may affect the response in assay including age, sex (Urlando and Heddle, 1990) and diet (Viragano et al, 1993).

Sanchez - Galan et al. (1998) have scored micronucleus number in kidney erythrocytes of wild Brown trout, Salmo trutta, caught in different fluvial ecosystems characterized by different levels of anthropogenic influences. According to them, brown trout samples from rivers with high anthropic influence possess significantly higher frequencies of micronuclei than fish sampled from less human

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influenced river. Ayllon and Garcia-Vazquez, (2000) have compared the sensitivity of European minnow (Phoxinus phoxinus) and mollie (Poecilia latipinna) to toxic heavy metals (cadmium and mercury) using micronucleus test in renal erythrocytes. They concluded that mollie is sensitive to both metals whereas minnow is not sensitive to mercury. Hoshinaa et al (2008) performed MN assay on erythrocytes of Oreochromis niloticus (perciformes, cichlidae) in order to evaluate the water quality of the Atibaia River, in an area that receives effluents discharge of a petroleum refinery and also to evaluate the effectiveness of the treatments used by the refinery. Yadav and Trivedi (2009) have reported that sub lethal exposure of heavy metals induces micronuclei in fish, Channa punctatus. Significant increase over and above negative control in the frequency of micronuclei was observed in fishes exposed to heavy metal compounds. Talapatra et al (2010) reported that metronidazole (MTZ), a nitroimidazole drug, is primarily used as an anti-protozoan or an anti-bacterial drug in human, although its genotoxic and carcinogenic effects have been widely reported, particularly in aquatic organisms. MTZ has induced micronuclei in the erythrocytes in Channa punctatus in laboratory conditions. Nuzhat and Shadab (2011) studied the effect of agriculture pesticide Malathion on Channa punctatus through micronucleus test. Several ecotoxicological characteristics of air breathing freshwater food fish Channa punctatus such as its wide distribution, availability throughout the year, easy maintenance in the aquaria/wet lab, ease of blood collection non-invasively and the presence of 32 well differentiated diploid chromosomes make this species an excellent model for toxicity studies (Kumar et al, 2010; Muranli and Guner, 2011) have reported the induction of micronuclei (MN) in erythrocytes of mosquito fish (Gambusia affinis). Fish were exposed to three different concentrations of lambda-cyhalothrin (LCT) ($1\times10\text{-}^{4}$µg/l, 2×10^{-4}µg/l, 4×10^{-4}µg/l) for periods of 6, 12, 24, and 48h. The MN and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) were evaluated to assess genotoxicity and cytotoxicity. The pesticide LCT has induced MN in erythrocytes of Gambusia affinis. The PCE/NCE ratio was also decreased after 24 and 48h treatments of 4×10^{-4}µg/1LCT. According to them, the LCT has genotoxic and cytotoxic potential on Gambusia affinis. Buker et al (2012) have studied the genotoxicity and putative mutagenic effects of benzene (BZN) in the erythrocytes of the electric fish, Apteronotus bonapartii (gymnotiformes, apteronotidae) using the micronucleus test (MN), under controlled laboratory conditions. Present study was undertaken to assess the genotoxic potential of cadmium chloride, arsenic, chromium and nickel in Channa punctatus.

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