LIPID PEROXIDATION AND DNA DAMAGE AS BIOMARKERS OF POLLUTION INDUCED OXIDATIVE STRESS (OS) IN FISH

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Abstract: The uses of maximum amount of agricultural and industrial chemicals are entering the aquatic environment and being taken up into tissues of aquatic organisms. Different types of pollutants induce a range of toxicity mechanisms, such as oxidative damage to membrane lipids, DNA, and proteins and changes to antioxidant enzymes. Free radical reactions and the production of toxic ROS are known to be responsible for a variety of oxidative damages leading to adverse health effects and diseases.

Though some literature is available on pollution induced oxidative stress but it is mostly based on mammalian studies. This assessment reviews current knowledge and advances in the understanding of such oxidative processes in aquatic organisms because of their sensitivity to oxidative pollutants and their potential for environmental toxicology studies. A search of literature was performed to collect the studies that measured the oxidative stress markers of pollution in fish. Studies were searched in Google scholar, Medline, Science direct, research gate, Pub Med, SCOPUS, Web of Science and other websites related to the subject from 1990 to May 2015.

Results indicate that escape of activated oxygen during active electron transport is the main source of ROS in man and higher animals. Another possibility is that multiple redox-active flavoproteins also contribute a small fraction to the overall production of oxidants under normal conditions. To maintain proper cellular homeostasis a balance must be struck between the production and consumption of ROS. It is concluded that measurements of lipid peroxidation and DNA damage both in nucleus and mitochondria can be used as potential contenders for general biomarkers of oxidative stress. However, these markers may be noticeable differently in the field than in results found in laboratory studies. Hence a multifaceted approach should be taken in field studies.

Keywords: Antioxidant, Environmental pollution, Fish, Oxidative Stress, Xenobiotics.

1.Introduction: Living organisms encounter an array of stresses during their constant interaction with environment [1]. Anthropogenic impacts on aquatic ecosystems are prevalent in developed and developing countries. Over extraction of freshwater, mainly for industries and agriculture has lead to major deprivation of rivers, lakes and aquifers. Environmentally stimulated stresses often activate the endogenous production of reactive oxygen species (ROS), most of which are generated as derivatives of mitochondrial respiration. Therefore regular exposure to toxicants may enhance ROS-mediated oxidative damage. More amount of agricultural and industrial wastes enter aquatic environment and being taken up by aquatic organisms induce multiple changes. Some of them directly enhance ROS formation whereas others act indirectly [2]. Oxygen is essential for efficient energy production in all aerobic organisms. Reactive oxidative species (ROS) are formed as natural derivatives of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis [3], [4]. But, due to environmental stress (UV, heavy metal pollution, pathogens, heat exposure) ROS levels can increase significantly and induce oxidative stress (OS) which may affect major damage to cell and tissue organization and causes neurodegenerative disorders in man and other animals [5], [6], [7], [8]. In human beings, oxidative stress is thought to be connected in the development various diseases [5], [7] including cancer [9] and myocardial infarction [10]. On the other hand, reactive oxygen species can be helpful, as they are used by the immune system as a means to attack and kill pathogens [11] Temporary oxidative stress with short duration may also be vital in prevention of aging by induction of a process named mitohormesis [12].

Therefore, aquatic ecosystem is a sink for many ecological pollutants which can be taken up by aquatic organisms leading to disturbing of prooxidant/antioxidant balance in fish [13],[14], [15]. So only the direct determining of the oxidative stress response in biological systems has become the most adequate tool for early warning in ecotoxicology studies [13].

2. Methodology: A systematic search of Pub Med, Scopus databases, Med line, Google Scholar, Science direct, Researchgate and the reference lists of all included studies and major relevant review papers was performed to obtain the data. To find out the appropriate articles, Pub Med was searched using the key words prooxidants, antioxidants, aquatic organisms and ROS in various combinations. Case-control studies with fish subjects were considered for

inclusion. The articles selected were published in English between January 1985 and December 2015.

3. Results and Discussion: Living beings have distinctive systems for defending themselves against the detrimental effects of activated ROS. Oxidative stress (OS) can result in oxidative damage to lipids, proteins, carbohydrates, and nucleic acids and is considered to happen when there is a difference in the prooxidant-to-antioxidant ratio in biological system. In majority cases, the anomalous production of ROS can result in major damage to cell structure [16]. The indication of exact symptoms known to occur particularly at oxidative stress is lipid peroxidation (membrane damage) DNA damage and accumulation of lipofuscin pigments were found in many aquatic animals exposed to contaminants [17] Therefore, examining the change in activity of antioxidant enzymes such as SOD, CAT, and GPx is believed as a valuable method of indicating the oxidative stress. In recent times, degree of difference expression of the genes encoding these enzymes has also been used to identify biological toxicity and/or to monitor the impact of chemical pollutants [18], [19].

Biomarkers of Lipid Peroxidation*:* Usually lipid peroxidation (LPO) or oxidation of poly unsaturated fatty acids is measured as a level of thiobarbituric acid reactive substances (TBARS). It has been employed most often to analyze the effect of pollutants [14], [15], [20], [21]. Lipid peroxidation (LPO) is the oxidative degradation of lipids and occurs due to oxidative imbalance. It is the process in which free radicals take electrons from the lipids in cell membranes and causes changes in the fluidity and permeability of cell membranes and impairs the activity of membrane-bound enzymes, resulting in cell damage [22]. Lipid peroxidation also leads to the production various aldehydes like malondialdehyde (MDA), 4- hydroxynonenal (HNE), and thiobarbituric acid reactive substances (TBARS). The elevated LPO in fish from heavily polluted field sites was observed [23]. For instance, Farombi, et al. [35] demonstrated high level of TBARS in the African catfish (*Clarias gariepinus*) from the Ogun River of Nigeria, located close to major industries. TBARS levels were significantly higher in the liver, kidney, gills and heart by 177%, 102%, 168% and 71% respectively compared to control fish. Similarly, higher levels of LPO products were found in the blood of three cichlid fish species (*Oreochromis niloticus*, *Tilapia rendalli*, and *Geophagus brasiliensis*) from the site of heavy metal pollution. [24] (Stoliar, O.B. and Lushchak, V.I., 2012). In another experiment Dorval et al, [25] showed elevated level of hepatic LPO products in white sucker (*Catostomus commersoni*) from the river sites that contaminated by agricultural chemicals in Québec (Canada). The killifish (*Fundulus heteroclitus*) inhabiting of the Elizabeth River also exhibited higher LPO as compared to the control fish [26]. Variations in the level of TBARS in a liver of common carp (*Cyprinus carpio*) were also noticed between fish from rural and industrial sites in Western Ukraine[27]

Several studies also demonstrate that TBARS level in fish exposed to various pollutants is significantly higher than in controls. Nwani, C.D et al [28] found elevated level of lipid peroxidation in the liver of C. punctatus in response to the exposure to herbicide atrazine due to increased production of ROS. Increased ROS production may be associated with the metabolism of herbicide leading to the peroxidation of membrane lipids of the hepatocytes. Likewise, earlier investigations have reported the induction of lipid peroxidation by pesticides such as deltamethrin [29] alachlor,[30] malathion, [31], endosulfan [32], butachlor [23], pollutants [33], toxic metals [34], sewage [35] and effluent in fish [36].

But in contrast, But, in contrast elevated levels of lipid peroxidation was not observed in the brain and liver of goldfish Crassius auratus exposed to sub lethal concentration of Roundup® [37]. Other case study by Pandey et al [38] shown the absence of differences in TBARS concentration in gills, kidney and liver tissues of fish (Wallago attu (Bl. & Schn.) from polluted and non polluted areas. Similar results were found in the hepatopancreas of carp from polluted site [39]. Even though differences observed in the activities of antioxidant the intensity of LPO was the same in the fish from the both sites, signifying a stronger antioxidant capacity of this organ. Likewise, TBARS did not show inter-site differences in eelpout (*Z. viviparous)* [40]. In the same way, despite the variations in the antioxidant enzyme activities, there was no significant difference in TBARS concentrations in the liver of labrid fish (*Coris julis*) [41]. The diverse responses in antioxidant enzymes level and TBARS concentrations may be due differences in the species, the time and duration of exposure, type and concentration of stressors.

Lipofuscins pigments are the end-products of LPO can be gathered in lysosomes as insoluble granules. The sign of these pigments in the lysosomes also can be used for the evaluation of the level of membrane LPO [42]. In our opinion, the evaluation of lipofuscin levels as more suitable characteristic of damage to lipids than TBARS. However, the corresponding studies with fish in the field are limited and connected exclusively histological studies which may not permit to assess the oxidative stress response exactly. For example, the histopathological investigations in freshwater fish, i.e. largemouth bass (*Micropterus salmoides*) and redbreast sunfish (*Lepomis auratus*), showed the marks of lipofuscin pigment accumulation in polluted sites [43].

DNA damage: In aerobic cells, the uncontrolled reactive oxygen species (ROS) which are generated as a by-product of mitochondrial activity can cause severe damage to cellular macromolecules, particularly the DNA [44]. It is very well known that there are close correlation between oxidative stress (OS) and DNA damage [45]. Pollution induced ROS can provoke oxidative damage of DNA, including strand breaks and base and nucleotide modifications, particularly in sequences with high guanosine content [46]. However, the application of these methods in fish is inadequate. In a study by Rodriguez-Ariza et al [47] found that 8-oxodG determination in chromosomal DNA was a potentially useful biomarker of oxidative stress caused by urban and industrial effluents in a fish (*Sparus aurata).* Jin, Y et al [48] have studied the effects of cypermethrin exposure on the induction of hepatic oxidative stress and DNA damage in adult zebra fish and found that even low concentration of pesticide can cause heavy DNA damage and gene expression. Similarly, Patel et al [49] reported cypermethrin induced DNA damage in the brain, liver, and kidney, of mice. A significant dose-dependent increase in DNA damage was observed in the brain cells and mid gut of Drosophila melanogaster exposed to low concentrations of cypermethrin [50].

4. Conclusions: The above outcome of this study implies that oxidative stress (OS) induced by various pollutants including heavy metals is a central issue in aquatic ecosystems. It also provides an insight into the role of reactive species in pollution induced toxicity. For that reason fish can be used as model organism for the study of pollution induced oxidative stress. However, the species specific forms of biomarkers and mechanisms of their action still need to be investigated. Chemically mediated pathways can affect antioxidant responses at different levels including transcriptional, translational and catalytic functions. In aquatic organisms such mechanisms remain largely unexplored in environmental monitoring programmes. Besides, molecular responses of antioxidants are often not analogous by expected biochemical changes or cellular effects. So, care is required when interpreting the outcomes of ecological pollutants. Finally it is concluded that cell stress biomarkers may serve as important tools for biomonitoring and development of risk assessment protocols.

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