CHAPTER 7

Oxidative Stress And Antioxidant Defense Mechanisms In Fishes

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Abstract

Aquatic organisms are always exposed to a variety of environmental pressures. These include heavy metals and pesticides as well as industry-based waste and climate-related changes that disrupt normal cellular stability. When they are exposed to these types of stressors, there is often an increase in production of reactive oxygen species (ROS), which, in turn, creates oxidative stress. Oxidative Stress is created when there are higher levels of ROS than the cellular antioxidant defense systems can neutralize. This imbalance can damage important cellular macromolecules like lipids, proteins, and nucleic acids, and thus affects their ability to properly perform certain physiological and metabolic functions. Fishes have evolved complex antioxidant defense systems that consist of both enzymatic and non-enzymatic elements that protect them from oxidative damage. Common antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST). There are also numerous examples of available non-enzymatic antioxidants such as reduced glutathione. These mechanisms are crucial to maintaining cellular redox balance in fishes that are subject to environmental toxins. In the realm of aquatic toxicology, oxidative stress biomarkers are commonly used as sensitive measures to evaluate the level of environmental contamination, as well as provide insight on the physiological health and ecological status of fish populations.

Keywords- *Oxidative stress, Reactive oxygen species, Antioxidant enzymes, Fish physiology, Aquatic toxicology, Environmental pollution*

Introduction

As a result of increased industrialization, agricultural operations, intense urban growth, and global climate change, aquatic ecosystems are more exposed to environmental stressors than ever before. Many contaminants are released into aquatic environments as a result of these activities (e.g., heavy metals, pesticides, industrial waste, hydrocarbons, and other xenobiotic pollutants). Fish, one of the major components of aquatic food webs and one of the most frequently used bioindicators, are especially sensitive to environmental disturbance. Fish exposure to contaminants disrupts normal physiological and biochemical functioning, which leads to metabolic imbalance and cellular injury (Lushchak, 2011). A key biochemical effect of fish being exposed to contaminants is the overproduction of reactive oxygen species (ROS). ROS are chemically reactive oxygen species that include superoxide anions, hydrogen peroxide, and hydroxyl radicals. ROS are produced naturally in the body through metabolic processes, particularly by the mitochondria during respiration. Under physiological conditions, ROS have a beneficial role in cellular signalling and in the immune response. However, when atmospheric conditions increase ROS production above what would normally occur, cellular oxidative stress will occur (Livingstone, 2001). Under the definition given by Halliwell & Gutteridge (2015), oxidative stress refers to the state of a biological system that has an excess of reactive oxygen species (ROS) and an insufficient capacity for oxidant defenses (e.g., antioxidant systems) to neutralize those excess ROS. In cells, too many ROS can damage vital cellular macromolecules such as lipids, proteins and nucleic acids. The result of lipid peroxidation is a disruption of the cellular membrane; the result of the oxidation of protein is disrupted activity of enzymes; and the result of damage to DNA may be mutations and/or improper cellular functions. These outcomes may ultimately affect growth, reproduction, immune system function, and/or the survival of fishes (Halliwell & Gutteridge, 2015). Fish have highly advanced antioxidant defense mechanisms that are able to protect their cells from oxidative damage by regulating redox homeostasis. Antioxidant defenses of fish consist of both antioxidants and other antioxidant systems. Some antioxidant enzymes that serve to detoxify ROS and therefore prevent oxidative injury are superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase (Birben *et al.*, 2012). Other antioxidants include reduced glutathione, vitamins C and E and carotenoids. These antioxidants aid in scavenging free radicals and protecting the integrity of cellular structure (Birben *et al.*, 2012).

Environmental monitoring and aquatic toxicology are increasingly using oxidative stress biomarkers as valuable assessment tools. Measuring the activities of antioxidant enzymes and the level of lipid peroxidation products found in the tissues of fish indicates the extent of exposure to pollutants and any physiological stress resulting from exposure to those pollutants. Thus, studying how fish respond to oxidative stress is a key element in understanding how pollutants are toxic, assessing the health of the aquatic ecosystem, and developing strategies designed to protect the environment (Valavanidis *et al.*, 2006).

Reactive Oxygen Species (Ros)

Reactive oxygen species (ROS) are molecules that contain an oxygen element and demonstrate a chemical reactivity that makes them play a fundamental role in cellular metabolism. ROS are produced as by-products of normal metabolic processes, specifically during normal mitochondrial respiration (i.e. through the electron transport chain). Under physiological conditions, ROS have various beneficial cellular roles including cell signaling and gene expression regulation as well as functioning as part of the immune defense response. But, when excessive ROS are generated, cellular homeostasis is disrupted leading to oxidative stress and resulting in injury to biological molecules and cellular structures (Lushchak, 2011). The most frequently occurring reactive oxygen species in biological systems are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), and singlet oxygen ($^1O_2$). Hydroxyl radicals are among the most highly reactive ROS produced inside cells and can result in injury to cellular lipids, proteins, and nucleic acids; superoxide anions are primarily produced in the mitochondrial electron transport system and hydrogen peroxide is produced as a result of enzymatic reactions via oxidases and superoxide dismutase (Birben *et al.*, 2012). Reactive oxygen species (ROS) are formed via multiple physiologically and biochemically based processes including but not limited to mitochondrial respiration, enzymatic oxidative reactions, immune system responses, and xenobiotic metabolism. In aquatic animals such as fish, exposure to different types of environmental stressors can cause a significant increase in the amount of ROS produced at the tissue level (e.g., heavy metals, pesticides, industrial pollutants, UV radiation, temperature change/variability, hypoxia (Halliwell & Gutteridge, 2015). Under normal physiological conditions, the level of ROS in an organism is tightly controlled through the use of antioxidant defence mechanisms that act to neutralise the effects of ROS and keep the cellular redox balance. However, if the production of ROS exceeds the antioxidant defence capabilities, oxidative stress occurs which may lead to lipid peroxidation, oxidative modification of proteins, and damage/deterioration of DNA in the tissues of fish (Valavanidis *et al.*, 2006).

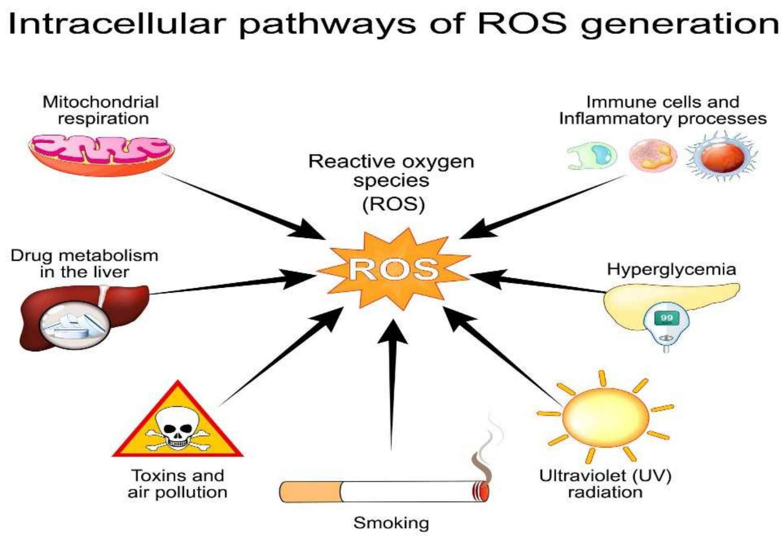

Figure:1 *Major reactive oxygen species including superoxide anion, hydrogen peroxide, hydroxyl radicals, and singlet oxygen.*

Sources Of Oxidative Stress In Fish

Fish experience oxidative stress due to the influence of many environmental and anthropogenic factors. Many aquatic ecosystems are contaminated by pollutants produced either from industrial processes, agricultural activities or urban habitats. These pollutants can disrupt cellular metabolism and induce the overproduction of reactive oxygen species (ROS), which results in oxidative damage to the tissues of fish (Lushchak, 2011).

1. **Heavy Metals**
   Heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb), and copper (Cu) are well-known inducers of oxidative stress in aquatic organisms. These metals accumulate in fish tissues through water and food chains and interfere with mitochondrial respiration and electron transport systems. As a result, excessive ROS are produced, which can damage cellular components. Heavy metals also inhibit important antioxidant enzymes, weakening the natural defense system of fish and promoting lipid peroxidation and protein oxidation (Halliwell & Gutteridge, 2015).
2. **Pesticides**

Agricultural pesticides including organophosphates, carbamates, and pyrethroids are widely used in crop protection and frequently enter aquatic ecosystems through surface runoff. These compounds disrupt metabolic pathways and mitochondrial function, resulting in increased ROS production. Pesticide exposure has been shown to alter antioxidant enzyme activity and induce oxidative damage in fish tissues (Livingstone, 2001).

3. **Industrial Effluents**

   Industrial wastewater often contains a mixture of toxic chemicals such as dyes, hydrocarbons, solvents, and heavy metals. Discharge of untreated effluents into water bodies can significantly increase ROS production in fish and impair antioxidant defense mechanisms (Valavanidis *et al.*, 2006).

4. **Environmental Stressors**

   Environmental factors such as temperature fluctuations, hypoxia, salinity changes, and ultraviolet radiation can also increase metabolic activity and ROS generation in fish, thereby contributing to oxidative stress (Sies *et al.*, 2017).

Mechanisms Of Oxidative Damage

Excessive production of reactive oxygen species (ROS) can severely damage various cellular components in fish, ultimately impairing normal physiological functions. When ROS levels exceed the antioxidant defense capacity, oxidative damage occurs in lipids, proteins, and nucleic acids, leading to cellular dysfunction and tissue injury (Halliwell & Gutteridge, 2015).

Lipid Peroxidation

ROS attack polyunsaturated fatty acids present in cellular membranes, resulting in lipid peroxidation. This process disrupts membrane integrity, alters membrane fluidity, and increases cellular permeability. Lipid peroxidation products such as malondialdehyde (MDA) are commonly used as indicators of oxidative damage in fish tissues (Lushchak, 2011).

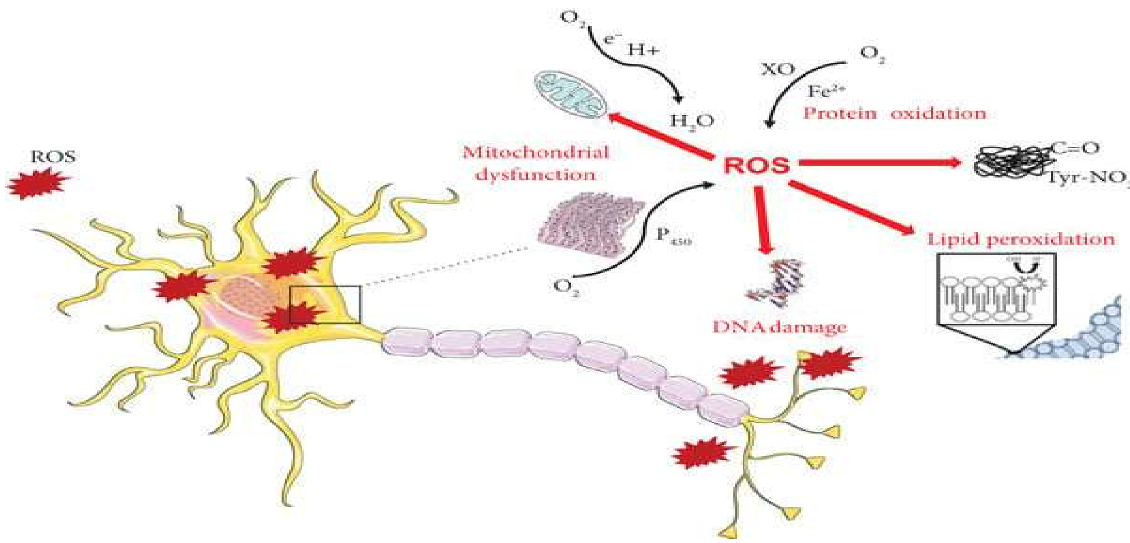

Figure:2 *Mechanism of lipid peroxidation caused by reactive oxygen species leading to cellular membrane damage.*

Protein Oxidation

Reactive oxygen species can oxidize amino acid residues in proteins, leading to structural modifications, enzyme inactivation, and loss of biological function. Oxidative modification of proteins can interfere with metabolic processes and cellular signaling pathways (Birben *et al.*, 2012).

DNA Damage

ROS can cause DNA strand breaks, base modifications, and chromosomal abnormalities. Such genetic damage may disrupt normal gene expression and cellular replication.

Apoptosis and Cellular Dysfunction

Severe oxidative stress may activate apoptotic pathways, leading to programmed cell death and tissue damage in fish organs.

Enzymatic Antioxidant Defense

Antioxidants which are produced by enzymes play a key role in protecting cells and the body from the damaging effects of oxidative stress (free radicals). Reactive oxygen species (ROS) can disrupt normal cell function and cause an imbalance between oxidants and antioxidants. By converting reactive molecules to non-reactive (less toxic) molecules, enzymatic antioxidants protect the cell from damage and allow for proper functioning of metabolic processes.

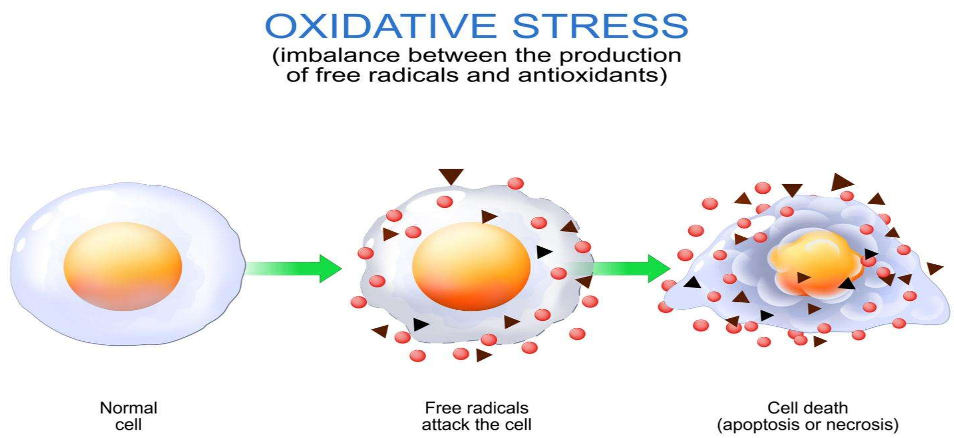

Figure: 3 *Antioxidant defense system showing the role of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in detoxifying ROS.*

- **Superoxide Dismutase (SOD)**
  Superoxide dismutase is one of the most important antioxidant enzymes present in fish cells. It converts superoxide radicals into hydrogen peroxide and molecular oxygen. By removing superoxide radicals, SOD acts as the first line of defense against oxidative stress and prevents the initiation of harmful free radical reactions.

- **Catalase (CAT)**
  Catalase is an important antioxidant enzyme that decomposes hydrogen peroxide into water and oxygen. Since hydrogen peroxide can generate highly toxic hydroxyl radicals, catalase plays a crucial role in preventing oxidative damage in fish tissues.

- **Glutathione Peroxidase (GPx)**
  Glutathione peroxidase reduces hydrogen peroxide and lipid peroxides into harmless products using reduced glutathione as a substrate. This enzyme protects cell membranes from oxidative degradation.

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- **Glutathione Reductase (GR)**

  Glutathione reductase regenerates reduced glutathione from its oxidized form, thereby maintaining the intracellular antioxidant pool.

- **Glutathione-S-Transferase (GST)**

  Glutathione-S-transferase participates in detoxification by conjugating toxic compounds with glutathione, facilitating their removal from cells.

Non-Enzymatic Antioxidants

Non-enzymatic antioxidants play an essential role in protecting fish cells from oxidative damage and complement the activity of enzymatic antioxidant systems. These antioxidants act by directly scavenging reactive oxygen species (ROS), preventing the initiation of free radical chain reactions, and maintaining cellular redox balance. They are present in various cellular compartments and work synergistically with antioxidant enzymes to maintain physiological stability in fish exposed to environmental stressors.

I. **Reduced Glutathione (GSH)**

   Reduced glutathione is one of the most important intracellular antioxidants found in fish cells. It acts as a powerful free radical scavenger and plays a crucial role in detoxifying reactive oxygen species. GSH also participates in maintaining cellular redox homeostasis and supports the activity of several antioxidant enzymes involved in oxidative stress defense.

II. **Vitamin C**

   Vitamin C, also known as ascorbic acid, is a water-soluble antioxidant that protects cellular components from oxidative damage. It neutralizes reactive oxygen species and helps regenerate other antioxidants such as vitamin E, thereby strengthening the antioxidant defense system in fish.

III. **Vitamin E**

   Vitamin E is a lipid-soluble antioxidant that protects cell membranes from oxidative damage. It prevents lipid peroxidation by stabilizing membrane lipids and interrupting free radical chain reactions.

IV. **Carotenoids**

Carotenoids such as astaxanthin and beta-carotene enhance antioxidant capacity and improve immune responses in fish, contributing to protection against oxidative stress.

Biomarkers Of Oxidative Stress In Fish

Oxidative stress biomarkers are widely used in aquatic toxicology to evaluate pollutant exposure.

Important biomarkers include:

Biomarker	**Function**
SOD	Superoxide radical detoxification
CAT	Hydrogen peroxide decomposition
GPx	Lipid peroxide detoxification
GST	Xenobiotic detoxification
GSH	Cellular antioxidant capacity
MDA	Indicator of lipid peroxidation

These biomarkers provide valuable information about the physiological condition of fish exposed to environmental pollutants.

Ecotoxicological Significance

Biomarkers of oxidative stress are useful tools used to measure the effects of pollution on aquatic life. They serve as an early warning of potential toxic pollution in aquatic systems before they cause visible physiological or ecological harm. They are also helpful for providing information on the presence and severity of environmental stressors, including heavy metals and pesticides, by measuring oxidative stress from antioxidants and damage to fish tissues. Because they have sensitive responses to changes in the environment and exposure to pollutants, fish are often used as bioindicators. By studying the activity of antioxidants and levels of lipid peroxidation (a type of oxidative stress) in fish, scientists can use them to assess the physiological state of fish and identify sub-lethal toxic effects from exposure to contaminants in aquatic environments.The study of oxidative stress biomarkers also supports efforts in risk assessment and environmental monitoring. Understanding the ways that pollutants induce oxidative damage in fish provides scientists with tools for assessing the overall health of aquatic systems and will allow them to develop appropriate strategies for managing pollution, conserving aquatic resources, and managing aquatic communities sustainably.

Conclusion

Oxidative stress represents one of the most important biochemical mechanisms responsible for toxicant-induced damage in fish. Exposure to environmental pollutants such as heavy metals, pesticides, and industrial chemicals significantly increases the production of reactive oxygen species (ROS) in fish tissues. Excessive accumulation of these reactive molecules can damage essential cellular components including lipids, proteins, and nucleic acids, ultimately leading to physiological disturbances, impaired metabolic functions, and reduced survival of aquatic organisms. Despite these harmful effects, fish possess efficient antioxidant defense systems that help counteract oxidative damage and maintain cellular homeostasis. These defense mechanisms include both enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, and non-enzymatic antioxidants like glutathione, vitamins, and carotenoids. Together, these protective systems play a crucial role in neutralizing reactive oxygen species and protecting fish cells from oxidative injury. Understanding the mechanisms of oxidative stress and antioxidant responses is essential for evaluating the impact of environmental contaminants on aquatic organisms. Biomarkers such as antioxidant enzyme activities and lipid peroxidation products provide reliable tools for environmental monitoring and aquatic toxicology studies. Future research should focus on identifying novel oxidative stress biomarkers, investigating natural antioxidant compounds, and developing effective strategies to reduce oxidative stress and protect aquatic ecosystems.

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