e-ISSN 2320 –7876 www.ijfans.org
Vol.12, Iss.1, 2023
 UGC CARE Listed (Group -I) Journal

A Comparative Study Of Malathion Induced Oxidative Stress And Defences In Intestinal Macrophages Of Channa Punctatus.

Raj Kusum Basumatary[1*]

[1*]Department of Zoology

***Corresponding Author:** Raj Kusum Basumatary
*Department of Zoology

ABSTRACT:
Malathion is an organophosphorous insecticide widely used in agricultural and non-agricultural purposes in India, Fishes were sampled, acclimatized and kept treated (0.5 ppm) with malathion and untreated with malathion under observation for 4 days. At day 4, lipid peroxidation activity and superoxide dismutase level increased significantly in treated group as compared to the control. On the other hand, glutathione peroxidase and glutathione reductase activity decreased on treatment with malathion. These results suggest that malathion induces oxidative stress, affects the antioxidant defense system and can cause physiological disturbances that reduce the health and survival of exposed fish.The aim of this study is to assess the adverse effect of malathion on Channa punctatus, in relation to oxidative stress leads to depletion of defences in intestinal macrophages.

Keywords: Channa punctatus**.** Malathion, Oxidative stress,

INTRODUCTION:

One of the most important causes of water pollution is the use of pesticides on farmland and tea gardens. Chemical pollutants from agriculture and industry are the main cause of pollution in rivers and water bodies near them. This is one of the most important environmental problems today. The broad spectrum organophosphate pesticide malathion is non-systemic. It was one of the 1950 developed organophosphate pesticides. Both agricultural and non-agricultural uses exist for malathion. The irrational use of organophophorous pesticides is one of the main causes of environmental pollution.
Reports showing that chemical exposure above a threshold can lead to an integrated stress response that can lead to immune suppression or immune activation have been factored into research interests. Fish are exposed to various pollutants dissolved in water or introduced into the food chain, so fish are not only prone to negative toxic health effects, but also accumulate pollutants. Fishes may therefore be used as bio-indicators of environmental contamination.

Fish tissues are endowed with an antioxidant defense system to protect them from oxidative stress caused by contaminants [6]. Elevated levels of contaminants can induce oxidative stress by generating highly reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radical and hydroxyl radicals that can oxidize proteins, lipids and nucleic acids, often leading to damage in cell stress or even cell death [7]. Organisms have developed several protective mechanisms to remove ROS before the detrimental effects occur in cell. Antioxidant enzymes such glutathione peroxidase (GPX), glutathione reductase (GR) and superoxide dismutase (SOD) are of great importance in oxidative stress to cope with free radicals leading several disturbances. Considering the above and the lack of sufficient information on the possible toxic effects of malathion on freshwater fish, the aim of this work was to evaluate its effect on the antioxidant profiles of *C. punctatus*.

MATERIALS AND METHODOLOGY:

Animals:

Freshwater fish *Channa punctatus*, of length 12 - 14 cm and weight 20.0 - 25.0 g, were obtained from local fish market. Fish of a single lot were used throughout the investigation. Prior to exposure, fish were kept for 15 days for acclimatization.

Exposure:

The toxicity tests were conducted in accordance with standard method. The fish were exposed to the sub lethal concentration (0.5ppm) of Malathion for 96 hrs and one batch was maintained as control i,e without pesticide and recorded the mortality rate of fishes. Sub-lethal toxicity of malathion on the intestinal macrophages of *Channa punctatus* was analyzed on the 4 day of exposure.

Isolation of intestinal macrophages:

Macrophage was then isolated from the cell suspension by the method of Secombes .

Superoxide dismutase (SOD) activity:

One unit of SOD activity was defined as the amount of enzyme that inhibited pyrogallol auto oxidation by 50%. Activity was expressed as protein U/mg.

Estimation of lipid peroxidation:

LPO activity was determined according to Utley et al. with some modifications [12].

Glutathione peroxidase (GPx) activity:

Total cellular GPx activity was determined by slight modification López et al. method.

Glutathione reductase (GR) activity:

Gluthathion reductase activity was determined by Carberg and Mannervile method.

Statistical Analysis:

Data were expressed as mean ± standard deviation. Data were analyzed using Student's t-test to determine significant change from control values. The level of significance was set at $P < 0.05$.

RESULTS:

Effect of malathion on superoxide dismutase released from intestinal macrophages:

Superoxide dismutase (SOD) release was found to increase from 0.019 ± 0.01 U/mg protein in control group to protein ($P < 0.05$) in malathion treated group. This suggests that malathion can over activate SOD activity, causing excess hydrogen peroxide to be produced, causing damage to the host cell.

Effect of malathion on lipid peroxidation in intestinal macrophages:

There was a significant increase in lipid peroxidation activity as it is evident from the results that showed increase of TBARS to 0.73 ± 0.137 n moles/hr ($P < 0.05$) in malathion treated group. The increased production of lipid peroxides means that malathion can actually disrupt the integrity of the plasma membrane, which is essential for cell viability, leaving cells vulnerable to damage.

Effect of malathion on glutathione peroxidase activity in intestinal macrophages:

Glutathione peroxidase activity was found to decrease to 3.6 ± 0.02 ($P < 0.001$) in malathion treated group, indicating the likely persistence of free radicals such as hydrogen peroxide that cause severe cell damage.

Effect of malathion on glutathione reductase activity in intestinal macrophages:

Glutathione reductase activity was found to decrease to 4.1 ± 0.01 ($P < 0.02$) in lead treated group indicating lead induced compromised antioxidant defense system in fish.

Organophosphate pesticides have broad spectrum of harmful effects in the living world. The pesticide malathion (an organophosphate) is being extensively used as dust, emulsion and vapor to control wide variety of insect pests under different conditions and reported to have a low toxicity to mammals and relatively high toxicity to fish [16]. It ultimately finds a way into aquatic habitats such as runoff from agriculture lands, rivers, lakes and ponds.

Fish have different defense mechanisms that prevent the effects of toxic substances. Pesticide accumulated in fish tissues may trigger redox reactions that generate free radicals especially reactive oxygen species (ROS). These highly reactive compounds may damage lipids, proteins, carbohydrates and nucleic acids and may induce morphological and physiological alterations in fish tissues. Oxidative stress occurs when the critical balance between oxidants and antioxidants is disrupted due to the depletion of antioxidants or excessive accumulation of ROS or both, leading to cellular damage. The SOD system provides the first defense against oxygen toxicity and represents a cellular defense mechanism to counteract toxicity of ROS. The increase in SOD activity in malathion treated fishes may be due to increased generation of reactive oxygen species (ROS). When the animal defenses are insufficient to neutralize ROS, oxidative damage may occurs and one of the most serious damages is membrane lipid peroxidation. The integrity of plasma membrane is essential for cell viability. The results demonstrate that malathion exposure induces production of high levels of lipid peroxides thus causing deleterious membrane damaging effect. Antioxidant enzymes that have often been studied as oxidative stress biomarkers link detoxification of ROS with the metabolism of reduced glutathione. These include glutathione peroxidase (GPx), an enzyme that removes hydrogen peroxide while simultaneously oxidizing reduced GSH to oxidized glutathione disulfide (GSSG) and glutathione reductase (GR), an enzyme that catalyzes the conversion of GSSG back to its reduced bioactive form and thus GSH/GSSG balanced. Thus, the present study shows a significant depletion of GPx and GR activity, indicating impairment of the detoxification and defense mechanisms of fish with low concentrations of malathion exposure making them vulnerable to environmental stressors.

Conclusion:

In the conclusion, this study demonstrates that sub-lethal concentrations of malathion are capable of bioaccumulation, altering the normal functional activity of the freshwater fish *Channa punctatus*. In addition, the association of oxidative stress, including variations in its antioxidant profile, suggests that exposure to malathion at low concentrations significantly impairs the defense system of *Channa punctatus* highlighting the vulnerability of aquatic organisms to even low levels of pesticide contamination.

References:

1. Al-Haj, M., Nasser, A., and Anis, A.(2005). Survey of pesticides used in Qat cultivation in Dhale and Yafe and their adverse effects. *J. Nat. Appl. Sci*. 9(1), 103-110.
2. Ghosh, T.K., and Chatterjee, S.K. (1989). Influence of Nuvan on the organic reservoirs of Indian fresh water murrel, *Channa punctatus. J . Environ. Biol*. 10, 93- 99.
3. Singh, R.N., Pandey, P.K., Singh, N.N., Das, V.K. (2010). Acute toxicity and Biochemical responses of common carp (*Cyprinus carpio(Linn*.) to an organophosphate (Dimethoate).*World Journal of Zoology*, 5(3), 183-188.
4. Tilak, K.S., Sattyavardhan, K., and Tathaji, P.B. (2003). Biochemical changes induced by Fenvelerate in the fresh water fish *Channa punctatus* . *J. Ecotoxicol. Environ.Monit*, 13, 261-270.
5. Wendelaar Bonga, S.E. (1997). The stress response of fish. *Physiological Reviews*, 77, 591-626.
6. Atli, G., and Canli, M. (2008). Responses of metallo thionein and reduced glutathione in a freshwater fish *Oreochromis niloticus* following metal exposures. *Environmental Toxicology and*

Pharmacology, 25, 33-38.
7. Nagalakshmi, N. (1998). Copper-induced oxidative stress in *Scenedesmus bijugatus*: Protective role of free radical scavengers. *Bulletin of Environment Contamination and Toxicology*, 61, 623-628.
8. Hinton, D.E. (1994). Cells, cellular responses, and their markers in chronic toxicity of fishes. In: Malins, D.C. and Ostrander, G.K., Eds., *Aquatic Toxicology*. *Molecular, Bio- Chemical and Cellular Perspectives*, Lewis Publishers, Bo- ca Raton, 207-239.
9. Lowry, O.H., Rosenbrogh, N.J., and Randall, R.J. (1951). Protein measurements with folin-phenol reagent. *J. Biol.Chem*, 193, 265-267.
10. Secombes, C.J. (1990). Isolation of salmoid macrophages and analysis of their killing activity. *Techniques in Fish Immunology*, 1, 137-155.
11. Marklund, S., and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47, 469-474.
12. Utley, H.C., Bernheim, F., and Hachslein, P. (1967). Effect of sulfhydryl reagent on peroxidation in microsome. *Archives of Biochemistry and Biophysics*, 260, 521-531.
13. López, T.M., Pérez, C.R., S, Cadenas., C, Rojas., and G. Barja. (1993). A comparative study of free radicals in vertebrates—II. Non-enzymatic antioxidants and oxidative stress. *Comparative Biochemistry and Physiology part B*, Volume 105, Issues 3–4, July–August 1993, Pages 757-763.
14. Carlberg, I., and Mannervik, E.B. (1975). Glutathione Level in Rat Brain. *Journal of Biological Chemistry*, 250, 4475- 4480.
15. Pritchard, J.B. (1993). *Environmental Health perspectives Journal Article Aquatic Toxicology Past, Present and Prospects*, 100, 249-257.
16. Edwards, C.A. (1973). *Environmental Pollution by Pesticides.* Plenum Press. London. New York.