



EFFECT OF DIAZINON ON ANTIOXIDANT SYSTEM OF FRESH WATER FISH, *CATLA CATLA*

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ABSTRACT

The present study was undertaken to evaluate the effect of Diazinon, an organophosphorous pesticide on oxidative stress biomarkers and lipid peroxidation formation in freshwater fish *Catla catla* for over 30 days. Significant induction of lipid peroxidation, superoxide dismutase and Catalase activity was found in liver, gill, kidney and muscle tissues of experimental fish. On the contrary, the activity of Glutathione – S – Transferase was found to be reduced in the fish exposed to diazinon when compared to control. The changes in the oxidative enzymes were more pronounced in with increase the pesticide exposure periods. The results suggest that the fish were under oxidative stress when exposed to Sub-lethal concentration of diazinon. It is proposed that measurement of antioxidant enzymes in fish tissues may prove to be useful in biomonitoring of exposure to aquatic pollutants.

KEYWORDS: Diazinon, an organophosphorous, Sub-lethal, biomonitoring, pollutants.

INTRODUCTION

India is the largest producer of pesticides in Asia and ranks 12th in the world. Heavy use of organochlorine insecticides has led to the dissemination of these pollutants throughout the global environment. Use of pesticide has become a necessary demon for developing countries like India where it is estimated that approximately 30% of its crop yield valued at Rs.60,000 crores are lost due to pest attack each year.^[1] Amongst others, organophosphorus pesticides (OPs) are the most commonly used pesticides in the world due to their quick degradation.^[2] Unfortunately, OPs lack target specificity and can cause severe, long lasting population effects on terrestrial and aquatic nontarget species, particularly vertebrates.^[3] Quick degradation is perhaps the reason why, irrespective of reports of health hazardous, developing countries especially, in the Asia-Pacific region, use these chemicals for agricultural and public health purposes.^[4, 5] It is one of the most commonly used organophosphate pesticides in developing countries.

Diazinon (IUPAC name: O,O-Diethyl O - [4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate, INN - Dimpylate), a colourless to dark brown liquid, is a thiophosphoric acid ester developed in 1952 by Ciba-Geigy, a Swiss chemical company (later Novartis and then Syngenta). It is a nonsystemic organophosphate insecticide formerly used to control cockroaches, silverfish, ants and fleas in residential, non-food buildings. Diazinon was heavily used during the 1970s

and early 1980s for general-purpose gardening use and indoor pest control.

Diazinon is a widely used toxicant in a various of organophosphorus pesticides (Rober and Hutson, 1998). Although the aquatic environment is not the targetone for the use of such pesticides the results of a number of monitoring studies have showed the apparition of diazinon and its metabolite, diazoxon, in surface waters (De Vlaming et al., 2000). Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish (Edsall, 1999). Changes in the biochemical blood profile mirror changes in metabolism and biochemical processes of the organism, resulting from the effects of various pollutants, and they make it feasible to study the mechanisms of the effects of these substances. The major biochemical retort to the effect of diazinon in fishes is the inhibition of enzymes. Hamm et al. (1998) also observed changes in carbohydrate metabolism in the eel, *Anguilla anguilla*. The glycogen contents in the liver and muscular tissue was significantly decreased while glucose and lactate concentrations in the blood were significantly increased. Present study aims to evaluate the acute effect of diazinon on oxidative enzymes of various tissues of *Catla catla* following the determination of LC₅₀ of diazinon in *Catla catla* for 96 hours by Vineela and Janardana Reddy (2014).

It is evident from the previous reports that Organophosphate pesticides, besides the inhibitory

function of AChE, also caused to change characteristics of oxidative stress⁴. Superoxide dismutase (SOD), whose substrate is free radical (superoxide anion; O₂⁻) catalyzes dismutation reaction resulting in the generation of hydrogen peroxide. This H₂O₂ is decomposed to water and molecular oxygen by the action of catalase. When the radical production overwhelms the endogenous levels decreased, they cause sustainable cell damage or cell death. All the major biomolecules such as lipid protein and nucleic acids may be assault by free radicals, but lipids are probably most susceptible.^[5]

The cells have different mechanism to attenuate and repaired damaged macromolecules. The primary defense is propounded by enzymatic and non enzymatic antioxidants which have been shown to scavenge free radicals and reactive oxygen species (ROS). The antioxidant enzymes, SOD, CAT and glutathione peroxidase (GPX) have been shown to be significantly affected by pesticides.^[6] Antioxidants belonging to the second route of defense including glutathione (GSH), vitamin C, Vitamin E (α -tocopherol acetate) and β carotene.^[7]

Organophosphate poisoning may persuade oxidative stress leading to generation of free radicals. Free radicals are formed which then increases oxidative destruction of lipids (lipid peroxidation), is a destructive interminable chain reaction, liberate malondialdehyde (MDA) as the final product.^[8] In view of this our study has intense on antioxidant parameters such as superoxide dismutase, catalase and oxidative stress lipid peroxides.

MATERIALS AND METHODS

The freshwater fish *Catla catla* was collected from and were acclimated to the laboratory conditions for about 15 days and were feed with planktonic materials and other food during the period of acclimation, water was replaced daily after feeding. The fishes were starved for 24 hr before exposure to avoid nutritional effects. LC₅₀ was determined before under taking the present investigations. The fish *Catla catla* of approximately same size and weight (12.0 $\pm$ 1.2g) were used for the experiment. The fishes were treated with 1/4th sublethal concentration of LC₅₀. The data statistically analyses by performing student 't' test.

Collection of the Blood samples: Collection of samples Blood samples were collected from control and organophosphate pesticide treated fish. The blood was immediately centrifuged at 3000 rpm for 15 minutes and the plasma separated. The cells were washed with normal saline and RBC's were subjected to lysis.

Catalase: Catalase activity was assayed by the method of Beers and Sizer.^[8] 100 mg of tissue homogenized in 1 ml of 0.1M phosphate buffer (pH -7.4). The reaction mixture consisted 2.9 ml of 0.059M H₂O₂ in 0.05M phosphate buffer (pH -7.0) and 0.1 ml of enzyme extract.

Catalase activity was calculated in terms of nmol. of H₂O₂ consumed min⁻¹ mg of protein⁻¹.

Lipid peroxidation: Lipid peroxidation was determined by the procedure of Utley et al.^[9] 100 mg of tissue was homogenized in 1 ml of 0.9% NaCl solution. To the extract 1ml of 20% TCA (chilled) was added and centrifuged at 3000 rpm for 30 min. The assay mixture contained 2 ml of 0.67% thiobarbitric acid and 1 ml of sample supernatant. The mixture boiled in water bath for 1 hr. The rate of lipid peroxidation was expressed as nanomoles of thiobarbitric acid reactive substance formed h⁻¹ g⁻¹ of tissue using a molar extinction coefficient 1.56 x 10⁵ M⁻¹ cm⁻¹.

Glutathione-S-transferase: Glutathione-S-transferase was determined by the Habig et al.^[10] method. The reaction mixture consists of 0.1 M phosphate buffer (pH -6.5), 1 mM CDNB, 1 mM reduced glutathione and 0.1 ml enzyme extract. The enzyme activity was calculated as μ mol. CDNB conjugate formed min⁻¹ mg of protein⁻¹ using molar extinction coefficient of 9.6x10³ M⁻¹cm⁻¹.

Superoxide dismutase: The activity of SOD was estimated by the method by Marklund and Marklund.^[11] To 2.2 ml of pyrogallol (C₆H₆O₂), 0.3 ml of enzyme extract was added and the colour developed was recorded at 420 nm by using spectrophotometer. The percent change in SOD activity was calculated as absorbance min⁻¹ mg of protein⁻¹.

Protein Estimation: Protein concentration in different samples was assayed by Lowry et al.^[12] method using Folin's reagent and BSA as standard.

Statistical analyses of data: The data obtained from this study were subjected to various statistical tools. The differences in the means ($\pm$ SEM) between groups were assessed using students't-test, Pearson's correlation and Levene's tests for equality of variance (SAS, 1988). A P-value of P < 0.05 was taken as significant.

RESULTS AND DISCUSSION

OP pesticide poisoning is predominantly a problem of developing countries like India. The impact of the organophosphates on fish revealed that besides AChE inhibition, there was alteration in peculiarity of oxidative stress.^[13] Concerning GSH content, the observed depletion in the glutathione GSH is considered as an early termination of diazinon induced oxidative stress as GSH content in this study is due to oxidation of GSH to glutathione disulfide GSSG by free radicals assembled by diazinon insecticide. Also, depletion of GSH may be allocated to detoxification process depending on glutathione-S- transferase (GST) enzymes which is one of enzyme system ingest GSH molecules as a substrate in the detoxification of OP insecticides to non-toxic products or by quickly irrevocable and very slowly turning over the insecticide.^[14] Augments in GSH levels

indicate a protective response in fish towards exposure to an oxidative-stress-induced diazinon stress.

GSH is a substrate of enzymes namely GPx and Glutathione-S- transferase. GSH/GSSG ratio is a very important indicator of the redox status of the cell.^[15] It has been observed that pesticides toxicity disturb this ratio, again confirming the presence of oxidative stress.

Our results suggest that pesticide poisoning influence on oxidative stress by depleting intracellular GSH and increasing ROS production. GSH is known to play a key role in modulating intracellular levels of ROS by scavenging free radicals to maintain the intracellular redox status. Organophosphate pesticides appear to disturb this key cellular pathway conceivably by disrupting mitochondria metabolism.^[16]

Table-1: Variations in oxidative enzymes and lipid peroxidation in tissues of fish exposed to sublethal concentration of Diazinon for over 30days.

Parameter	Tissues	Control	Days of Exposure Periods			
			3Days	7 Days	15Days	30Days
Glutathione-S-transferase (nmol CDNB conjugates-1 min ⁻¹ mg protein)	Liver %change	1.24±0.01	0.93±0.001	0.74± 0.001	0.65 ±0.001	0.43 ±0.002
	Gill %change	0.67±0.01	0.54±0.02	0.35±0.01	0.29±0.02	0.18±0.01
	Kidney %change	0.435± 0.005	0.379±0.003	0.324±0.002	0.299±0.001	0.283±0.001
	Muscle %change	1.13±0.03	0.89±0.03	0.63±0.02	0.41±0.01	0.33±0.02
Superoxide dismutase (Unit- ¹ mg protein)	Liver % change	0.83±0.04	0.76±0.02	0.99±0.03	1.11±0.03	1.27±0.04
	Gill % change	0.57±0.02	0.48±0.02	0.67±0.01	0.81±0.02	1.050.03
	Kidney % change	0.61±0.04	0.55±0.01	0.77±0.02	0.95±0.03	1.17±0.03
	Muscle % change	0.24±0.02	0.18±0.01	0.29±0.01	0.49±0.02	0.62±0.02
Catalase (nmol H ₂ O ₂ consumed-1min-1mg protein)	Liver % change	173±0.02	187±0.02	168±0.01	157±0.01	137±0.01
	Gill % Change	215±0.03	235±0.04	197± 0.03	179±0.02	151±0.02
	Kidney % change	134±0.01	154±0.02	125±0.03	108±0.02	0.84±0.02
	Muscle % Change	158±0.03	169±0.04	143±0.03	121±0.02	103±0.02
Lipid Peroxidation (nmol TBARS formed-1h-1g tissue)	Liver % Change	87.9±0.03	113.6±0.04	138.1± 0.03	157±0.02	179±0.03
	Gill % Change	36.26±0.03	43.18±0.03	55.30±0.02	69.49±0.03	84.250.03
	Kidney % change	68.36±0.02	78.02±0.03	83.72±0.02	91.54±0.03	100.41±0.04
	Muscle %change	55.13±0.04	65.26±0.04	78.15±0.03	89.62±0.03	99.16±0.02

In the aquatic environment, despite the presence of enhanced antioxidant defence systems, increased / decreased levels of oxidative destruction occur in organisms exposed to contaminants which stimulate the production of ROS increased production of ROS and subsequent oxidative damage has been associated with pollutant-mediated mechanisms of toxicity in fish liver (Livingstone, 2001). Proteins constitute also a target for oxidative damage with subsequent adaptations of their functions. In flounders, living in contaminated waters with xenobiotics, increased levels of oxidised proteins were reported from polluted sites.^[17]

OP pesticide poisoning is primarily a problem of developing countries like India. The role of oxygen free radical (OFR) has been well established in many chronic disorders. The signification of the implication of OFR in acute condition like OP pesticide toxicity has not been investigated so far.

Catalase is a ubiquitous enzyme present in cells of aerobic organism. It educate hydrogen peroxide to molecular oxygen and two molecules of water.^[18] In this respect, it is spellbinding to record that catalase activity was decreased. The decrease in catalase activity could be due to the flux of superoxide radicals, which have been

reported to inhibit CAT activity.^[19] The same decreased activity of CAT was noticed in tissues of *Channa punctatus* Bloch exposure to Deltamethrin.^[20]

Catalase plays an important role in the decomposition of H_2O_2 to water. Due to altered glutathione redox ratio and a subsequent increased in reduced glutathione levels, the glutathione mediated detoxification process may also be afflicted. CAT, an enzyme that transforms hydrogen peroxide into hydrogen and oxygen, plays an antioxidant role and its activity increasing in acute poisoning surrender to oxidative stress. SOD has been reported to participate in the reduction of superoxide anion to less reactive molecular oxygen and hydrogen peroxide species. The H_2O_2 can be further degraded by CAT or by glutathione peroxidase in the presence of reduced glutathione. Compound with other antioxidative enzyme, SOD and CAT are more vulnerable and efficient and reciprocate more rapidly in the face of oxidative stress.^[21] In the present study it is obvious from the results that in the Diazinon treated fish CAT activity may be due to a flux of superoxide radicals, which have been shown to suppress CAT activity.^[22] Under acute oxidative stress, toxicity of pesticides may overwhelm antioxidant defense.^[23]

A significant increase in lipid oxidation marker may specify the susceptibility of lipid molecules to reactive oxygen species and the elongation of oxidative damage imposed on these molecules. Lipid peroxidation levels significant increase in different tissues of *Catla catla* may be due to antioxidant enzyme activities that are up regulated upon oxidative stress in different tissues of the fish in increasing order. Similar observations of antioxidant enzyme levels in fish such as *Geophagus brasiliensis* are also reported in response to oxidative stress were made earlier (Lenartova *et al.*, 1970). Maintenance of high constitutive levels of antioxidant enzymes like superoxide dismutase and catalase is crucial to prevent oxyradical mediated lipid peroxidation.^[24]

Relating to the effect of sub lethal intoxication of *Catla catla* to diazinon on oxidative stress parameters, the data exhibited in Table -1 declared a significant elevation of serum reduced glutathione content (GSH), serum SOD activity and in lipid peroxidation biomarker (MDA) after diazinon insecticide exposure against control groups at $p < 0.05$. Associating with oxidative stress biomarkers, the findings in our experiment are coincident with that acquired by^[25] which supporting the intoxication.

Following treatment with Diazinon the thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation (MDA) level decreased significantly as well as activity of SOD and CAT were also significantly inhibited thereby indicating attenuation of oxidative stress inflicted by OP pesticide poisoning. Generation of oxidative stress and sequential lipid peroxidation by OP pesticides could be due to high concentration of

polyunsaturated fatty acids in the cells, lipid peroxidation is a major resultant of the free radicals mediated injury. Two broad outcomes of lipid peroxidation are structural damage of cellular membranes and generation of oxidized products, some of which are chemically reactive and may covalently transform cellular macromolecules. These reactive products are thought to be the major effector of tissue damage from lipid peroxidation.^[26] It is concluded that there was an appreciable increase in lipid peroxide levels indicating an extensive oxidative stress in OP pesticide treatment.

We conclude that the evaluation of oxidative stress biomarkers in freshwater fish, *Catla catla* could provide a useful indicator of pollution of water bodies. The results also indicated that the antioxidant defense components namely, LPO, SOD, CAT, GPx, GST and GSH are sensitive parameters that could provide useful biomarkers for the assessment of contaminated aquatic ecosystems. The relationship between the degree of deficiency of antioxidant defense and lipid and protein oxidation propose that these parameters could also be used as biomarkers. Hence, as part of an effort to continue monitoring the ecological health of the estuary Island and their inhabitants and to establish environmental safety, this type of study represents an important tool because of its unique approach to ensure the correlations between metal concentration in water and their bioaccumulation in aquatic species along with the evaluation of contaminant impact on stress biomarkers.

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