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Comparative study of tissue lipid and protein peroxides as oxidative stress markers in sublethal malathion exposed Indian Catfish *Heteropneustes fossilis*

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ABSTRACT

Effect of organophosphorus pesticide malathion on fish in laboratory condition was studied. The level of toxicity and stress was observed with protein and lipid peroxides as probe parameters. Healthy matured specimens of H. fossilis were exposed with malathion in two sub-lethal concentrations (0.1 and 0.2 ppm) for 30 days. Lipid and protein peroxides contents were estimated in five different tissues- muscle, gill, brain, liver and kidney. Malathion was observed to be effective in producing oxidative stress in the fishes even in very low doses. Lipid peroxide (LPO) and protein peroxides (PPO) levels were significantly higher in all the tissues of pesticide-exposed groups with different exposure time compared to control group.

Keywords: *Malathion, Fish, Lipid peroxide, Protein peroxide*

INTRODUCTION

Indiscriminate use of pesticides in agricultural operations adversely affects the aquatic environment to a great extent. This poses a great danger to freshwater organisms including fishes. This affects not only the growth and survival of fishes[7,10,14] but also other aquatic organisms, which serve as food for different animals. Malathion is one of the most selective organophosphate pesticides used for the control of pests on vegetables, field crops, fruits and domestic animals. Though the organophosphate pesticides may disappear rapidly from the body either by hydrolysis or elimination, long term and repeated exposure to these organophosphate pesticides have cumulative effects on proteins and different enzymes[3,16].

The toxicity of a large number of xenobiotics depends on their conversion to free radicals, which initiate the processes of lipid peroxidation (LPO) and protein peroxidation (PPO) in cell membranes. Free radical production and lipid peroxidation are complex and deleterious process, which are closely related to toxicity[6]. Because of the potential for ROS to damage tissues and cellular components such as membrane lipids and proteins, oxidative stress has become a topic of significant interest in aquatic toxicology[8,9,13]. The lipid peroxidation process is considered to be an important indicator of membrane damage[11], which leads to cell death[12]. Besides lipids, proteins are also easily attacked by reactive oxygen species directly or indirectly through lipid peroxidation. Protein radicals can be rapidly transferred to other sites within the protein infrastructure. This can result in further modification of enzyme activity. So, lipid and protein oxidative damage can be used as marker of xenobiotic toxicity. However, convincing evidences are scanty regarding the effect of OP pesticide malathion in relation to the alteration of LPO and PPO contents of the fish. Therefore, the present study is aimed to investigate the effect of sublethal doses of malathion on lipid and protein peroxides in Indian catfish *Heteropneustes fossilis.*

MATERIAL AND METHODS

Healthy, sexually mature specimen of *H. fossilis* of same age group and average weight (15 – 18 gm) were procured from the local fish farm. The fishes were kept in glass aquariums (containing 100 liters of water in each aquarium) in the laboratory at water temperature of 25°C - 27°C. They were acclimatized for 15 days in the experimental water in laboratory condition before the commencement of the experiment. The water of the aquarium was changed daily and fishes were fed with goat liver ad libitum. The OP pesticide malathion (50%EC) was obtained from the local market (manufactured by Assam Chemical Industries, Bongaigaon, Assam,India). A pilot experiment carried out to find out the LC_{50} value of malathion by Probit analysis[4] and LC_{50} for 96 hours was found to be 9.98 ppm. Two sub lethal concentrations (0.1 ppm and 0.2 ppm) were prepared by using standard technique[1].

After acclimatization in the laboratory condition the fishes were divided into two groups – Control group (group-I) consisting of normal healthy fishes and Experimental group (group-II) which was again divided into two subgroups – group II A and group II B. Sub group II A was treated with 0.1 ppm malathion and II B was treated with sub lethal concentration of 0.2 ppm malathion.

Lipid peroxides (LPO) were estimated in both control and malathion treated groups from the day of treatment at 24 hrs, 4^{th}, 7^{th}, 15^{th}, 25^{th} and 30^{th} day. The assay of LPO was done by the thiobarbituric acid (TAB) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of lipids[15]. The muscle, gill, brain, liver and kidney tissues were homogenized in ice-cold 0.15 M phosphate buffer (pH 7.4, 10% wt/volume) and the homogenates were centrifuged at 5000 rpm for 20 minutes. The amount of MDA formed was measured by using the molar extinction coefficient for MDA at 530 nm[15].

Protein peroxides (PPO) were estimated in all five tissues of control and malathion exposed groups in different time intervals like the LPO. The assay of PPO was done by following the method of Gay *et al.*[5]. For this tissue homogenate was mixed with equal amount of 10% cold trichloroacetic acid (TCA) and precipitate was collected by centrifugation. The collected precipitate was washed three times with 10% cold TCA and resuspended in 25mM sulphuric acid for the determination of hydroperoxides. The sulphuric acid suspended precipitate was then mixed with 5 mM ferrous ammonium sulphate and equal amount of xylenol orange and incubated in room temperature for 30 minutes. The resultant ferric ions are measured as the xylenol orange complex at 560 nm. The results were analyzed statistically using student's 't' test.

RESULTS

The results obtained in the present investigation are summarized in Table 1,2 and 3. The present analysis revealed increased level of mean LPO and PPO in all the tissues of malathion-exposed groups of fishes in comparison to control group. On exposure to sub-lethal malathion in doses of 0.1 ppm, the peroxidation of lipids in muscle is significantly affected from the initial period of exposure for 24 hours till the end of the experiment up to the 30^{th} day. The initial increase in lipid peroxide (LPO) on exposure for 24 hours is marginally significant with 34% increase above the control group, which is sustained with a uniform gradient up to the 25^{th} day of the experiment.

Table I: Lipid peroxide content (n mol/mg tissue) in different tissues of control and malathion exposed groups of *H. fossilis*

Table I: Lipid peroxide content (n mol/mg tissue) in different tissues of control and malathion exposed groups of *H. fossilis*

Tissues	Groups	Exposure periods					
		24hrs	4th day	7th day	15th day	25th day	30th day
Muscle	Control	0.38 ± 0.04	0.42 ± 0.04	0.37 ± 0.03	0.44 ± 0.03	0.40 ± 0.03	0.35 ± 0.03
	0.1ppm MT	0.51* ± 0.06	0.70*** ± 0.05	0.75*** ± 0.04	0.97*** ± 0.12	1.25***± 0.10	0.78*** ± 0.07
	0.2ppm MT	0.68*** ± 0.04	0.83*** ± 0.07	1.05*** ± 0.07	1.23*** ± 0.07	0.90***± 0.06	0.92*** ± 0.06
Gill	Control	1.50 ± 0.14	1.65 ± 0.13	1.6 ± 0.11	1.42 ± 0.10	1.58 ± 0.10	1.52 ± 0.11
	0.1ppm MT	1.52 ± 0.16	2.55*** ± 0.15	2.34** ± 0.17	2.64*** ± 0.20	2.97***± 0.25	2.63*** ± 0.19
	0.2ppm MT	2.01** ± 0.08	2.40*** ± 0.13	3.12*** ± 0.13	2.82*** ± 0.09	2.51***± 0.12	2.34** ± 0.11
Brain	Control	2.67 ± 0.09	2.72 ± 0.11	2.62 ± 0.09	2.74 ± 0.12	2.58 ± 0.10	2.65 ± 0.12
	0.1ppm MT	2.74 ± 0.13	2.83 ± 0.12	3.56*** ± 0.14	3.63*** ± 0.10	3.78***± 0.18	3.34*** ± 0.13
	0.2ppm MT	2.71 ± 0.13	2.94 ± 0.12	3.42*** ± 0.13	3.82*** ± 0.14	3.43***± 0.15	3.21* ± 0.15
Liver	Control	1.40 ± 0.09	1.46 ± 0.07	1.38 ± 0.08	1.56 ± 0.09	1.54 ± 0.09	1.42 ± 0.08
	0.1ppm MT	1.62 ± 0.10	2.03*** ± 0.09	2.14*** ± 0.14	2.35*** ± 0.11	2.17** ± 0.17	2.24*** ± 0.11
	0.2ppm MT	1.57 ± 0.08	2.34*** ± 0.13	2.53*** ± 0.09	2.62*** ± 0.11	2.75*** ± 0.10	2.14*** ± 0.11
Kidney	Control	1.69 ± 0.08	1.74 ± 0.10	1.56 ± 0.09	1.76 ± 0.10	1.48 ± 0.08	1.60 ± 0.10
	0.1ppm MT	1.71 ± 0.08	2.23** ± 0.10	2.40*** ± 0.13	2.95*** ± 0.16	2.88*** ± 0.15	2.64*** ± 0.15
	0.2ppm MT	1.64 ± 0.11	1.84 ± 0.11	2.56*** ± 0.14	2.72*** ± 0.12	3.12*** ± 0.11	2.86*** ± 0.11

N= 20, data are mean ± S.E.M., *p<0.05, **p<0.01, ***p<0.001, Here, MT= Malathion treated

Table 2: Protein peroxide content (n mol/mg tissue) in different tissues of control and malathion exposed groups of *H.fossilis*

Tissues	Groups	Exposure periods					
		24hrs	4th day	7th day	15th day	25th day	30th day
Muscle	Control	0.59 ± 0.05	0.52 ± 0.04	0.50 ± 0.04	0.46 ± 0.04	0.58± 0.05	0.54 ± 0.04
	0.1ppm MT	0.58 ± 0.04	0.42 ± 0.07	0.45 ± 0.04	0.59* ± 0.05	0.80**± 0.04	0.62 ± 0.06
	0.2ppm MT	0.56 ± 0.04	0.53 ± 0.03	0.61 ± 0.03	0.84*** ± 0.04	0.95***± 0.06	1.18*** ± 0.06
Gill	Control	1.28 ± 0.08	1.16 ± 0.09	1.22 ± 0.07	1.30 ± 0.08	1.19 ± 0.06	1.34 ± 0.09
	0.1ppm MT	1.23 ± 0.09	0.92* ± 0.07	1.28 ± 0.08	1.67* ± 0.14	1.52*± 0.10	1.58 ± 0.18
	0.2ppm MT	1.47* ± 0.06	1.59*** ± 0.06	1.92*** ± 0.07	2.12*** ± 0.12	2.48***± 0.13	2.35*** ± 0.11
Brain	Control	0.83 ± 0.06	0.87 ± 0.07	0.91 ± 0.08	0.93 ± 0.06	0.85 ± 0.06	0.80 ± 0.06
	0.1ppm MT	0.82 ± 0.07	1.12* ± 0.09	1.43*** ± 0.09	1.54*** ± 0.12	1.31***± 0.08	1.32*** ± 0.11
	0.2ppm MT	0.80 ± 0.06	0.98 ± 0.06	1.28** ± 0.06	1.74*** ± 0.08	1.52***± 0.07	1.41*** ± 0.08
Liver	Control	1.12 ± 0.07	1.10 ± 0.06	1.02 ± 0.06	1.16 ± 0.07	1.20 ± 0.06	1.18 ± 0.06
	0.1ppm MT	1.22 ± 0.06	1.06 ± 0.07	1.21 ± 0.10	1.63** ± 0.12	1.97*** ± 0.10	1.98*** ± 0.15
	0.2ppm MT	0.97 ± 0.07	1.34* ± 0.09	1.41** ± 0.09	2.12*** ± 0.12	2.20*** ± 0.09	2.32*** ± 0.12
Kidney	Control	1.11 ± 0.09	1.02 ± 0.07	1.08 ± 0.06	1.14 ± 0.08	0.98 ± 0.07	1.18 ± 0.06
	0.1ppm MT	0.94 ± 0.08	0.86 ± 0.07	1.35* ± 0.09	1.42* ± 0.11	1.21* ± 0.06	2.04*** ± 0.08
	0.2ppm MT	1.01 ± 0.06	0.98 ± 0.07	1.21 ± 0.06	1.64*** ± 0.08	1.96*** ± 0.10	1.72*** ± 0.09

N= 20, data are mean ± S.E.M., *p<0.05, **p<0.01, ***p<0.001, Here, MT= Malathion treated

Table 3: Showing the Percent deviation of mean values of LPO and PPO contents of malathion exposed groups from the control group of *H. fossilis* in different days of exposure

Tissues	Groups	Parameters	Exposure periods					
			24hrs	4th day	7th day	15th day	25th day	30th day
Muscle	0.1ppm MT	LPO	34.21	66.67	102.70	120.45	212.50	122.85
		PPO	-1.69	-19.23	-10.0	28.26	37.93	14.81
	0.2 ppm MT	LPO	78.94	97.62	183.78	179.55	125.0	162.85
		PPO	-5.08	1.92	22.0	82.61	63.79	118.52
Gill	0.1ppm MT	LPO	1.33	54.55	46.25	85.92	87.97	73.03
		PPO	-3.91	-20.68	4.91	28.46	27.73	17.91
	0.2 ppm MT	LPO	34.30	45.45	95.0	98.59	93.0	53.95
		PPO	14.84	37.07	57.38	63.08	108.40	75.37
Brain	0.1ppm MT	LPO	2.62	4.04	35.88	32.48	46.51	26.04
		PPO	-1.20	28.74	57.14	65.59	54.12	65.0
	0.2 ppm MT	LPO	1.50	8.09	30.53	39.42	32.95	21.13
		PPO	-3.61	12.64	40.66	87.09	78.82	76.25
Liver	0.1ppm MT	LPO	15.71	39.04	55.07	50.64	40.91	57.75
		PPO	8.93	-3.64	18.63	40.52	64.17	67.8
	0.2 ppm MT	LPO	12.14	60.27	83.33	67.95	78.57	50.70
		PPO	-13.39	21.82	38.24	82.76	83.33	96.61
Kidney	0.1ppm MT	LPO	1.18	28.16	53.84	67.61	94.59	65.0
		PPO	-15.32	-15.69	25.0	24.56	23.47	72.38
	0.2 ppm MT	LPO	-2.96	5.75	64.10	54.55	110.81	78.75
		PPO	9.0	-3.92	12.04	43.86	100.0	45.76

N= 20, data are mean ± S.E.M., *p<0.05, **p<0.01, ***p<0.001, Here, MT= Malathion treated

The changes in the LPO status in muscle with a dose of 0.2 ppm malathion are similar, showing a significantly higher response (p<0.01) than that of 0.1 ppm exposed group of experiment upto the 15th day. Lipid peroxidation of gill tissue in response to malathion exposure is enhanced from the onset of the experiment and is retained throughout the period, with a significant decline (p<0.01) from the maximum peak attained during the period of 1 to 2 weeks of exposure towards the end of the experiment on the 30th day. The increase in LPO level can be correlated with the concentration of malathion. Exposure to malathion in 0.2 ppm concentration shows early enhancement of LPO in gill tissue (p<0.01). Likewise, the peak increase of LPO by 98% above the base line is also attained earlier with a higher dose of malathion. In liver, the LPO content was significantly increased from the 4th day of exposure of malathion in both the groups and exhibiting highest value on 30th day in 0.1 ppm exposed group which was about 57.75% higher (p<0.001) than the control group. In 0.2 ppm treated group maximum increase of LPO was observed on 7th day, which was about 83.33% higher than the control group. Similarly in brain, the mean values of lipid peroxides increase from 4th day of treatment than the control group in 0.1 ppm treated group and it showed highest amount on 25th day which was about 46.51% higher than the control group. In 0.2 ppm exposed group highest amount of lipid peroxide was observed on 15th day and then it decreases gradually till 30th day of treatment but mean values were still higher than that of the control group. Similar results were observed in kidney tissue where maximum LPO level was observed on 25th day in 0.2 ppm malathion exposed group.

The protein peroxide (PPO) contents of all the tissues of malathion exposed groups are increased than the control group except the initial period of exposure. In the initial phase there is an apparent reduction of protein peroxidation on malathion exposure in all the tissues except brain.

This initial period of decrease in protein peroxidation is pronounced with 0.1 ppm concentration of malathion exposure and extends only up to the 4th day then it is reversed with enhancement of PPO during the subsequent period of experiment. However, this observed initial reduction is significant only in gill tissue ($p<0.05$). With 0.2 ppm of malathion concentration this initial period of reduced protein peroxidation is absent with only an apparent reduction in kidney tissue, which is not statistically significant ($p>0.05$). After this initial period of reduced protein peroxidation up to the 4th day of malathion exposure, the succeeding period of experiment is marked with persistent findings of significantly elevated protein peroxidation in all the tissues up to the period between 15th to 25th days followed by recession of peroxidation towards 30th day. During the period of elevated peroxidation the general trend of response with the two different concentration of malathion is similar and is more pronounced with the higher concentration of 0.2 ppm except in brain tissue where there is only an apparently lower response with the 0.2 ppm of concentration.

DISCUSSION

The peroxidation of lipid in different tissues on exposure to sublethal concentration of malathion at different time intervals during the period of study show a general increase in LPO contents with advancement of malathion exposure which is followed by a gradual decline toward the end of the experiment on 30th days after exposure irrespective to the concentrations of 0.1ppm and 0.2 ppm. During the study, it has been observed that out of the five tissues, muscle is the tissue most affected by peroxidation and brain is the least affected tissue. The lipids of gill, liver and kidney tissues are peroxidised moderately occupying an intermediate position, liver being at the center of the peroxidation affected tissues. The maximum involvement of muscle lipids may be due to a relatively weak free radical scavenging system in the muscle tissue. The minimum peroxidation of brain lipids even with maximum peroxidizable lipids may be due to the protected position of the brain exerted by the blood-brain barrier. It is also observed that a tissue with the least amount of peroxidised lipid responds by forming the highest amount of LPO and the tissue with highest amount of peroxidised lipid responds with formation of a minimum amount of LPO to malathion exposure.

The trend of protein peroxidation in different tissues under malathion exposure is observed to be relatively more persistent than the peroxidation of lipid on corresponding periods of exposure which is more pronounced with the higher dose of 0.2 ppm malathion (Table.2). The phase of gradual decline of LPO towards the terminal part of the experiment is delayed in case of protein peroxidation(PPO), which may be due to the relatively lower turnover rate of structural tissue proteins than the structural lipids that are more susceptible for peroxidation, by free radicals.

Generation of free radicals associated with insecticide poisoning as reported by various workers[2,17], the toxic effects of malathion observed with increased level of tissue peroxides in the present study is thought to be due to the result of pesticide induced free radical generation.

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