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Effects of Malathion on Retinoids and Carotenoids Reserves of Freshwater Fishes *Heteropneustes fossilis* and *Polyacanthus fasciatus*

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Abstract

The malathion, an organophosphate pesticide has shown strong pesticidal activity on retinoid and carotenoids reserves of freshwater fishes *Polyacanthus fasciatus* and *Heteropneustes fossilis*. The present study deals with the effect of sublethal dose of malathion on the concentration of retinoids reserves of liver and scales of the fishes *H.fossilis* and *P.fasciatus*. The LC_{50} value of malathion to the freshwater catfish *H.fossilis* at 96h exposure period is found as 28ppm and for the fish *P.fasciatus* the LC_{50} value at 96h exposure period is found as 5ppm. The decrease in retinoid and carotenoid level of both the fishes were observed during 96 hours in treated group compared with control.

Keywords: *Retinol . Dehydroretinol . β-carotene . Malathion*

1. Introduction

Since time immemorial the bright external colouration of animals, plants and fish in particular has attracted the attention of biologists and aquaculturists1,2,3. It has been known that the magnificent colouring particles of ornamental fishes are due to the presence of a lipid class of molecules known as carotenoids which provide the red, yellow, orange or green colouration of the fish. These lipid soluble pigments colour the fish integuments, muscle and gonads of the fish 4,5,6,7. During metabolism, the biogenesis of the carotenoid pigment and its conversion and absorption into various retinoids depend upon several environmental and nutritional factors factors such as water quality, monsoon, temperature,pH, presence of xenobiotics,etc.8,9,10,11.

For ensuring food security for the growing population all over the world, enhanced production from the agricultural field has become very important. To augment the production from agriculture, various kinds of pesticides have been used in agriculture to eradicate unwanted insects and control disease vectors.12 Organophosphorus compounds (OPs) are most common type of insecticides.13 But wide and uncontrolled use of Organophosphorus (OPs) pesticides over the years has raised serious concern about its effect on the environment. Malathion (ML) [O, O-dimethyl-S-1, 2-di-(ethoxycarbamyl) ethylphos-phor–dithioate] which is commonly used to control mosquitoes in the aquatic environment, tends to be overused and, by nature of its application method, can cause extensive contamination to aquatic environments. The consequences of contamination can be severe especially to aquatic animals14. This pesticide seems to be more toxic to insects and fish than mammals. According to its chemical structure, malathion is classified as an organophosphate pesticide15.

The extensive use of malathion on land may be washed into surface water and can adversely influence or kill the life of aquatic organisms and other higher animals. Aquatic organisms, particularly fish, are highly sensitive to malathion13.

The accumulation of pesticides in aquatic organisms and non-target animals such as fish, can result in increased susceptibility to diseases, reduced reproductive capabilities and altered growth rates16.

Many studies have been done to determine the acute toxicity of malathion in numerous species of fish17-21 .In present experiment, a calculated amount of malathion is prepared in tap water and fishes were exposed in order to see any effects on the retinoids and carotenoids levels of fishes.

2. Materials and Method

2.1 Collection of fish

Live specimens of adult freshwater catfish *Heteropneustes fossilis* (both sex, body wt 25-45 g) and *Polyacanthus fasciatus* (both sex, body wt 3-6 g) were collected from local market (Guwahati) and acclimatized to laboratory conditions for a period of 15 days in large glass aquaria, previously washed with potassium permanganate to free the walls from microbial infection. Water was changed daily and the fishes were fed with the aquarium feed as referred [9].

2.2 Solvents and Chemicals

The OP pesticide malathion (50%e.c.) is procured from local market which is manufactured by Assam Chemical Industries, Bongaigaon (Assam). Light petroleum ether (b.p. 40-60^0 C, 60-80^{o}C), L.R. grade was obtained from British Drug Houses (India), Glaxo Laboratories (India) Ltd. The solvent was dried over pure calcium chloride and distilled twice before used. Diethyl ether was supplied by Alembic Chemicals Works Co.Pvt. Ltd., Boroda. It was made peroxide free by distilling it over reduced iron. Chloroform was supplied from B.D.H. Chemicals Division, Glaxo Laboratories (India).Absolute ethanol was procured from Bengal Chemical and Pharmaceutical Works Ltd. Other solvents like acetone, acetic anhydride used were obtained from BDH, Laboratory Chemicals Division, Glaxo Laboratories (India) Pvt. Ltd. Different authentic retinoid samples, such as retinol,dehydroretinol, ß-carotene, .were obtained from Hoffman La Roche, Basel, Switzerland, BASF, Germany and Roche Co. Ltd., India. .

2.3 Determination of physicochemical characteristics of the tap water

The physico chemical characteristics of tap water were analysed following the methods mentioned in ANONYM[22] and found as

1.Temperature-28^0 ±2^0

2.pH---7.2 ±0.3

3.Dissolved oxygen—7.9±0.7mg/l

4.Total hardness—63.4ppm

2.4 Preparation of Stock solution of Malathion

A stock solution of toxicant was prepared and few concentrations from stock solution were prepared as per the dilution technique[23]. 5 gms of Malathion were dissolved in 50ml acetone to form a Stock solution from which required volumes were added to the jars to obtain the appropriate dose.

2.5 Determination of LC_{50} Value for malathion

LC_{50} value of malathion in experimental condition determined after following the method of Finney.[24]

2.6 Experimental procedure

For determination of the 96h LC_{50} value for malathion, a batch of ten acclimatized fish was exposed to different doses of malathion. Each batch of fish was kept in rectangular glass aquaria of 30 liter capacity containing tap water.The doses chosen were 20,21,22, 23,24,25,26,27,28,29 and 30ppm for *Heteropneustes fossilis* and 1,2,3,4,5,6 and 7ppm for *P.fasciatus*. Preliminary experiments with smaller number of fish indicated that the LC_{50} dose was somewhere between 25 and 29ppm for *Heteropneustes fossilis* and between 4 and 6ppm for *P.fasciatus* . The required quantity of malathion was drawn directly from the stock solution prepared earlier using a variable micropipette. Additional volume of acetone was added to these solutions, whenever required, to keep the acetone concentration in each jar the same(0.5ml/l).Control groups, each having ten fish kept in tap water containing acetone (0.5ml/l) was run concurrently. All solutions (control and test) were renewed daily and dead fishes were immediately removed. At different exposure periods (24,48,72 and 96h),the mortality of fish was observed. The concentration at which 50% survival/mortality occurred in malathion treated fishes was taken as the median lethal concentration (LC_{50}) for 96h. After 96h of exposure the data obtained was subjected to Finney's probit analysis method[24] to determine LC_{50} value. The experiment was continued for 30 days.

2.7 Estimation and Extraction of retinoids

After 30 days both the control and pesticide treated fishes were sacrificed and the liver and scales were dissected out and the pigments were isolated following the procedure referred from earlier studies[1,2,3]. The lipids were extracted through light petroleum (40-60°C) ether using anhydrous sodium sulphate.

2.8 Extraction of carotenoids and vitamin

A

Lipids from the livers and the scales of the fish were extracted through light petroleum (40-60°C) ether extract using anhydrous sodium sulphate[5, 25]. The extraction efficiency was tested by following a parallel method followed after Folch *et al.*,[26] . 200mg/lit of BHT was added to Folch solution or light petroleum ether which acted as antioxidant. It was found that both light petroleum and Folch solution showed similar extraction efficiency. However, in the present study, light petroleum (40-60°c) is used and retinyl propionate and β -apo-8[1] -carotenoic acid ethyl ester (CAEE) were used as internal standards.

The liver oil was extracted with light petroleum until the extract was colourless and gave no colour with $SbCI_3$) reagent. The combined extracts were filtered and the solvent removed by distillation under reduced pressure at 40°C. The last traces of the solvent were removed *in vacuo* and the oil preserved until further used or saponified under reflux for 10 minutes with methanolic solution of KOH (10% wt/ vol.). Vitamin A was extracted thrice with peroxide-free diethyl ether. The etheral extract was freed from alkali, dried over anhydrous sodium sulphate and the solvent was removed by distillation under reduced pressure. The saponicate was either dissolved in known volume of light petroleum ether or in HPLC solvent for estimation.

2.9 Estimation

The carotenoids and vitamin A extracts were estimated using HPLC technique in the Analytical Nutrition and Chemistry Division of National Institute of Nutrition (ICMAR) Hyderabad. The extracts were sealed under nitrogen and HPLC analysis was made. The HPLC system included a liquid chromatograph (various model 5000) and integration (No.4270), an inject (Rheodyne model 725) with a 20µl loop and a various wave length detector.

2.10 HPLC procedures

HPLC system (waters) with column 300mm x 3.9 mm Nova -Pack C_{18} (4 mm) and a Guard -Pak precolumn module (water 5) were used. Standard carotenoid and retinoids samples (5.0 mg) were dissolved in 100 ml toluene: methanol (1: 1) containing 500 mg BHT (butylated hydroxy toluene)/ litre for producing 50µg/ml standards. These standard stock solution is stable and could be preserved at -20°C for 4 months. These were further diluted with the mobile phase to give working standards.HPLC grade solvents were degassed by vacuum filtration prior to use and water double distilled.Both retinoids and carotenoids were separated using HPLC grade solvents, acetonitrile: dichloromethane : methanol: water: propionic acid (71:22:4:2:1, v/v) as mobile phase with the flow rate of 1.0 ml/minute in the first 10 minute run, detection of carotenoids pigments was performed at 450 nm and dehydroretinol in 352 and retinol in 326 nm. All the other HPLC procedures were followed after Guillou *et al*[27].

2.11 Statistical Analysis

All the data obtained during the period of investigation are statistically analysed after Sokal and Rohlf [28]. The level of significance between two sets of data are calculated according to students t-test. Probability i.e. P value at 5 percent or lower for two sets of data are taken as significant.

3. Results

The LC_{50} value for 96h exposure was found as 28ppm for *H.fossilis* and 5ppm for *C.fasciatus*. The mean retinoids and carotenoids levels of liver and scales of both the fishes *P. fasciatus* and *H. fossilis* showed significant decrease during 96 hours in treated group compared with control.

3.1 Effect of LC_{50} value of malathion on retinoids and carotenoids of fish *P. fasciatus*:

In LC_{50} dose of malathion treated condition it is found that the fish *P. fasciatus* shows the mean values for retinol, dehydroretinol, liver carotenoids and scale carotenoids concentration as 12(±1.5)µg/g, 9(±0.5)µg/g, 145(±7)µg/100g and 85(±5)µg/100g respectively while in control condition it is found as 28(±0.5)µg/g, 23(±1.5)µg/g, 164(±5)µg/100g and 120(±12)µg/100g respectively.

3.2 Effect of LC_{50} value of malathion on retinoids and carotenoids of fish *H. fossilis*:

In LC50 dose of malathion treated condition it is found that the fish *H. fossilis* shows the mean values for retinol, Dehydroretinol and liver carotenoids concentration as 2(±0.5)µg/g, 42(±5)µg/g and 65(±2)µg/100g respectively while in control condition it is found as 15(±0.5)µg/g, 65(±5)µg/g and 95(±5)µg/100g respectively.

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4. Tables and Figures

Table 4.1 Effect of LC_{50} dose of malathion on the retinoids and carotenoids concentration of different species of fish.

Fish and No	Liver Retinoids		Carotenoids	
(Control and treatment *)	Retinol (µg/g)	Dehydroretinol (µg/g)	Liver (µg/100 g)	Scales(µg/100g)
P. fasciatus				
n = 5 (Control)	28 (±0.5)	23 (±1.5)	164 (±5)	120 (±12)
n = 5 (LC_{50}dose)	12 (±1.5)	9 (±0.5)	145 (±7)	85 (±5)
H. fossilis				
n = 5 (Control)	15 (±0.5)	65 (±5)	95 (±5)	-
n = 5 (LC_{50}dose)	2 (±0.5)	42 (±5)	65 (±2)	-

Mean Values of the number of fishes shown ±SD and significant (P<0.05) when T - test was applied between the control and treated groups.

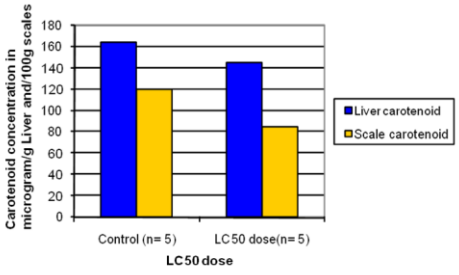

Fig 4.1: Effect of LC_{50} dose on Carotenoid concentration of *Polyacanthus fasciatus*

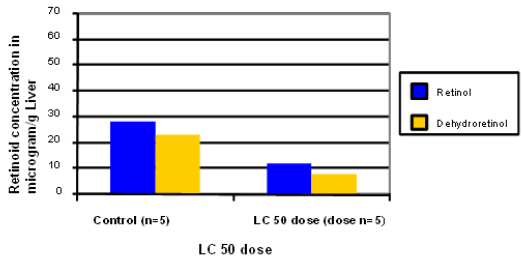

Fig 4.2: Effect of LC_{50} dose on Retinoid concentration of *Polyacanthus fasciatus*

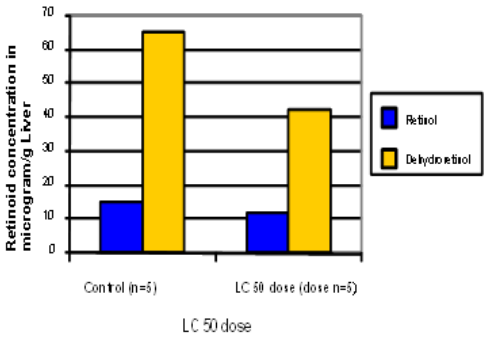

Fig 4.3: Effect of LC_{50} dose on Retinoid concentration of *Heteropneustes fossilis*

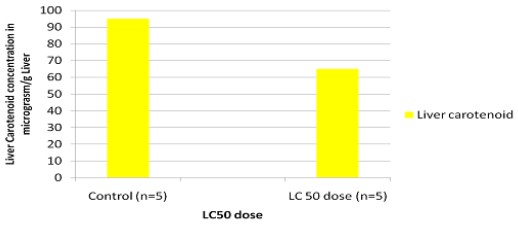

Fig 4.4: Effect of LC_{50} dose on Carotenoid concentration of *Heteropneustes fossilis*

4. Discussion

Retinoids resrves of fishes are controlled by various factors such as hormones, nervous control, nutritional status, overall physiological responses and environmental factors as well as its synthesis. Besides these, changes of season, presence of xenobiotics, age, sex and geographical isolation are responsible for the occurance, physiological actions of retinoids[79]. Temperature, salinity and internal factors such as reproductive physiology also influence the lipid content of freshwater organisms. Generally the present results indicated the toxic nature of the insecticide malathion on the retinoid and carotenoid of fishes.

The result of the present study showed that when the fishes were exposed to malathion, there have been a significant impact on the mobilization as well as concentration of carotenoid molecules. Fishes dwelling in malathion treated water showed significantly lesser amount of retinoid and carotenoid concentration, whereas controlled or normal water fishes show greater amount of pigmentation with high amount of retinoid and carotenoids. The present study derives support from the studies of Goswami[17], who reported that fishes treated with the malathion for 25 days show

decreasing amount of its carotenoid concentration which impacts highly on fish pigmentation. Srinivas *et al*.[29] has showed decreased lipid content in *T. mossambica* on exposed to atrazine. Gradual depletion in lipid content of liver and muscle when exposed to malathion was analysed by Mishra *et al*.[30].

It is found that lipid content of fish reduced with increasing concentration of pollution (Amudha *et al*.[31]). They reported that reduction in lipid content might be due to utilization of lipid as a source of energy during stressful condition. Gupta[32] has also showed that lipid content decreased in various tissues of *Channa punctatus* with increasing

concentration of vegetable oil factory efficient .Choudhary *et al*.[33] reported that the effect of malathion on the behavior and body composition of the *Heteropneustes fossilis* and found the water and lipid contents of the whole body and ovary decreased as compared to control.

It has been found elevated level in lipid on lamellidens marginalis, a freshwater bivalve exposed to mercury in monsoon season (Bano and Hasan[34]). Patil and Kulkarni (1995) found that when exposed to pesticide summach fish *Channa punctatus showed* the reduction in lipid content. Tazeen *et al*.[35] observed decline in total lipid content when the cat fish *Mystus vittatus* exposed to pesticide nuvan. Tantarpale *et al*.[36] also reported that decrease in total lipid content might be due to utilization of lipid during the toxic stress. Tazeen *et al*.[35] found the decline in total lipid content in different organs of cat fish *Mystus vittatus* exposed to the pesticide nuvan. They mentioned the reduction of total lipids is possibly due to the excessive lypolysis and subsequently used for synthesis of glucose.

From the above discussion it is clearly indicated that malathion is toxic to fresh water fish *H.fossilis* and *C. fasciatus*. The sublethal dose of organophosphorous pesticide malathion significantly altered retinoid and carotenoid levels in both the experimental fishes. It is concluded from present investigation that malathion even at sublethal concentration causes considerable changes in the lipid content of fishes *H.fossilis* and *C.fasciatus*.

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