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ORIGINAL ARTICLE

PROTECTIVE ROLE OF VITAMIN-C AGAINST MALATHION TOXICITY ON CERTAIN BIOCHEMICAL PARAMETERS IN LIVER OF FRESH WATER FISH

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ABSTRACT

The present study has been conducted to understand the protective role of vitamin C on malathion toxicity on certain biochemical parameters in the liver of *Clarias batrachus* for a period of 50 days. Five groups of 10 fish each were exposed separately to malathion 0.71ppm solution prepared in tap water. Each group was exposed for 6, 12, 25, 35 and 50 days to one of the following treatments: Group I: Control group, fish were reared in dechlorinated tap water and fed on a commercial diet (32% protein). Group II: Control group, fish were reared in tap water and fed on a commercial diet (32% protein) supplemented with 500 mg vitamin C/kg diet. Group III: Fish were exposed to 0.71mg/l of malathion and fed on a commercial diet (32% protein). Group IV: Fish were exposed to 0.71mg/l of malathion and fed on a commercial diet (32% protein) supplemented with 500 mg vitamin C/kg diet. The biochemical parameters analysed include total protein, glucose, glycogen and total lipid. All the parameters showed a non – significant variation among group I and II. In malathion exposed group (Group III), the value decreased significantly at all stages of exposure. The ascorbic acid supplementation enhances fish tolerance to environmental stress and reduces malathion toxicity in liver of the fish *Clarias batrachus.*

Keywords: liver, malathion toxicity, protein, glucose, lipid, *Clarias batrachus*

1.INTRODUCTION

The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. In India pesticides are one of the major classes of toxic substances for management of pest in agricultural sectors and control of insect vectors of human disease. Among pesticides, malathion (*O*-dimethyl-S1-2-di(ethoxycarbonyl)- ethyl phosphoro- dithioate) is an organophosphorous insecticide widely used in agriculture and houses for the control of disease vectors. It is a major source of environment poisoning in developing countries (WHO, 2003). Toxicological tests have shown that malathion affected central nervous system, immune system, adrenal gland, liver and blood. Moreover,the assessment of protein, glucose, glycogen and lipid contents can be considered as a diagnostic tool to determine the physiological phases of organism. In this context, the present investigation has been designed to get the information regarding sublethal toxic effects of malathion and the ameliorative role of Vitamin – C on biochemical parameters of liver of the freshwater fish *C. batrachus.*

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2.MATERIALS AND METHODS

The fresh water fish *Clarias batrachus* 15 ± 2 cm length and 36± 2 g weight were collected locally from Annamalainagar, Cuddalore district, Tamilnadu, India, were brought to the laboratory and kept in a tank size of 60 x 30 x 30 (l x b x h) cm, filled with tap water for acclimatization for about two weeks. During the acclimatization the fish were fed with minced goat liver on every alternate days. Water in the tank was renewed, three or four times in a week and aerated to ensure sufficient oxygen supply. For the fish used in experiments, feeding was stopped two days before the start of the experiments to reduce the quantum of excretory products in the tank. Prior to the commencement of the experiment the median lethal concentration (LC_{50}) for 96 h was calculated by trimmed Spearman Karber method (Hamilton *et al.*, 1972) and was found to be 7.1 ppm at 95% confidence limit.

Sublethal studies are helpful to assess the response of the test organism under augmented stress caused by the insecticide. For the analysis of sublethal toxicity five groups of 10 fish each were exposed separately to malathion (0.71ppm; 10% of 96 h LC_{50} value) solution prepared in tap water. The experimental medium was prepared by dissolving malathion (0.71ppm) in tap water having dissolved oxygen 6 ppm, pH 7.5, water hardness 40.44 mg/L and water temperature 28 ± 2 °C (APHA, 1992). Each group was exposed to 30 liter of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaria containing tap water without the addition of malathion as controls. Feeding was allowed in the experimental as well as

control groups everyday for a period of 3 h before the renewal of the media throughout the tenure of the experiment. Four groups of 10 fish each were exposed to one of the following treatments: Group I: Control group, fish were reared in tap water and fed on a commercial diet (32% protein). Group II: Control group, fish were reared in tap water and fed on a commercial diet (32% protein) supplemented with 500 mg vitamin C/kg diet. Group III: Fish were exposed to 0.71mg/l of malathion and fed on a commercial diet (32% protein). Group IV: Fish were exposed to 0.71mg/l of malathion and fed on a commercial diet (32% protein) supplemented with 500 mg vitamin c/kg diet. After the expiry of 6, 12, 25, 35 and 50 d of exposure five fish each from the respective experimental and control groups were sacrificed. Liver was excised along with control groups and were subjected to biochemical analysis. The total protein contents in the tissues was estimated by the method of Lowry et al. (1951), lipid content was estimated by the semi micro determination method of Pande et al. (1963), the estimation of glucose and glycogen was carried out following the methods of Kemp and Kits Van Heijhigen (1954).

3.RESULTS

Total Protein

The control liver recorded 86.52 mg/ g of protein. The value showed a non-significant variations among group I and II. In malathion exposed group (Group III,), the value decreased at all stages of exposure. The values are 76.18,75.21,66.19,46.18 and 44.16 mg/g of protein. But in group IV (malathion+ Vit. C), the value decreased significantly as 78.16,77.18,75.16,74.10 mg/g of protein for 6,12,25,35, and 50 days of exposure (Table 1 and Fig. 1).

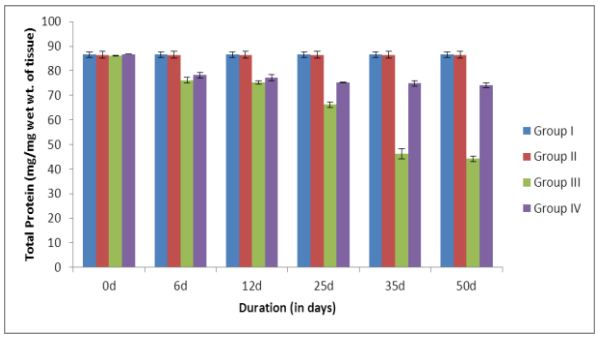

Fig.1. Showing the variations in quantity of total protein (mg/g wet weight of the tissue) in the liver of *C. batrachus* in different experimental groups for 6, 12, 25, 35 and 50 days.

Glucose

The control liver registered 5.51 mg/g of glucose. The value showed a fecreasing trend as 5.62,4.61,3.50,2.69,2.42 and 1.95 mg/g of glucose for 6,12,25,35 and 50 days (Table 1 and Fig.2) in Malathion exposed group (Group III). In group IV, the value decreased upto 50 days as 5.18,5.06,4.95,4.62,4.16 and 4.00 mg/g of glucose.

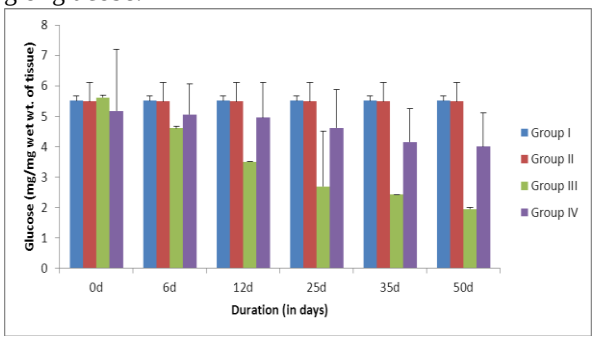

Fig.2. Showing the variations in quantity of glucose (mg/g wet weight of the tissue) in the liver of *C. batrachus* in different experimental groups for 6, 12, 25, 35 and 50 days.

Glycogen

The liver of control fish recorded 24.12 mg/g of glycogen. On exposure to malathion the value decreased non-significantly as 24.16,21.12,20.10,16.12 and 14.14 mg/g of glycogen, but after 50 days of exposure, the value decreased. But the addition of Vit.C with malathion the group IV also showed similar trend as 24.18,23.10,22.16,21.16,21.19 and 20.16 mg/g of glycogen respectively for 6,12,25,35 and 50 days (Table 1 and Fig.3)

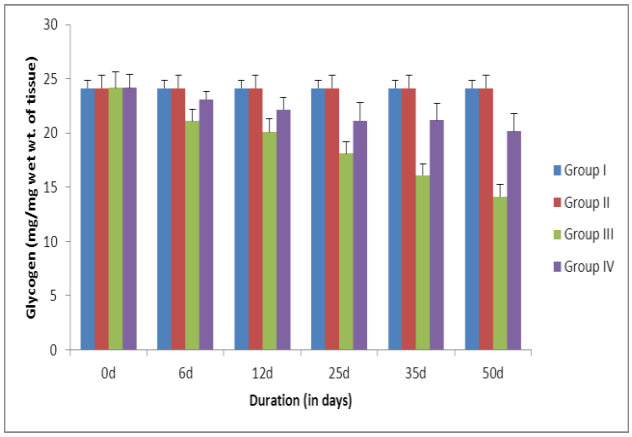

Fig.3. Showing the variations in quantity of glycogen (mg/g wet weight of the tissue) in the liver of *C. batrachus* in different experimental groups for 6, 12, 25, 35 and 50 days.

Total Lipid

The control liver recorded 36.12 mg/g of total lipid. The value shoed a non-significant variations among group I and II. In malathion exposed group (Group III), the value decreased significantly form 6 day to 50 day. The values are 36.10,34.14,32.16,30.10,26.18 and 23.12 mg/g of total lipid. Similar change was also recorded in Group IV (malathion + Vit.C) as 35.15,35.12,34.22,34.19 and 33.00 mg/g of total lipid after 6,12,25,35,and 50 days (Table 1 and Fig.4)

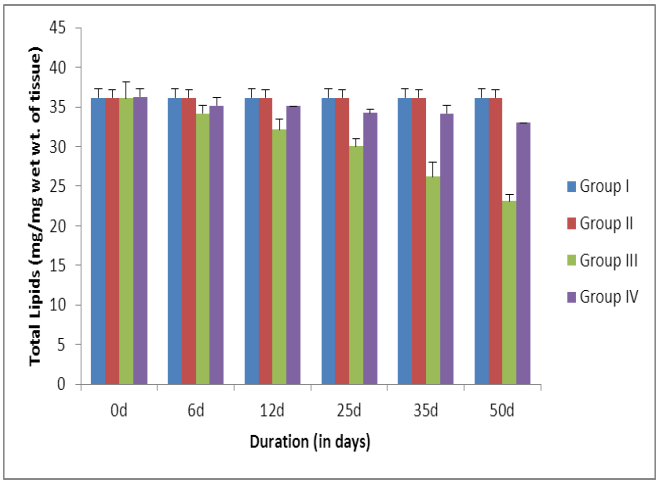

Fig.4. Showing the variations in quantity of total lipids (mg/g wet weight of the tissue) in the liver of *C. batrachus* in different experimental groups for 6, 12, 25, 35 and 50 days.

Parameters	Experimental Groups	Duration (in days) 0d	6d	12d	25d	35d	50d
Total Protein (mg/mg wet wt. of tissue)	Group I	86.52 ± 1.01	86.52 ± 1.00	86.52 ± 1.21	86.52 ± 1.01	86.52 ± 1.01	86.52 ± 1.00
	Group II	86.48 ± 1.32	86.48 ± 1.32	86.48 ± 1.32	86.48 ± 1.32	86.48 ± 1.32	86.48 ± 1.32
	Group III	86.12 ± 0.15	76.18 ± 1.15*	75.21 ± 0.65*	66.19 ± 1.14*	46.18 ± 2.15*	44.16 ± 1.10*
	Group IV	86.69 ± 0.01	78. 16 ± 1.00^{NS}	77.18 ± 1.12*	75.16 ± 0.18*	74.89 ± 1.16*	74.10 ± 1.10*
Glucose (mg/mg wet wt. of tissue)	Group I	5.51 ± 0.15	5.51 ± 0.15	5.51 ± 0.15	5.51 ± 0.15	5.51 ± 0.15	5.51 ± 0.15
	Group II	5.49 ± 0.62	5.49 ± 0.62	5.49 ± 0.62	5.49 ± 0.62	5.49 ± 0.62	5.49 ± 0.62
	Group III	5.62 ± 0.08	4.61 ± 0.05 ^{NS}	3.50 ± 0.02*	2.69 ± 1.82*	2.42 ± 0.01*	1.95 ± 0.06*
	Group IV	5.18 ± 2.02	5.06 ± 1.00 ^{NS}	4.95 ± 1.15*	4.62 ± 1.25*	4.16 ± 1.10*	4.00 ± 1.12*
Glycogen (mg/mg wet wt. of tissue)	Group I	24.12 ± 0.75	24.12 ± 0.75	24.12 ± 0.75	24.12 ± 0.75	24.12 ± 0.75	24.12 ± 0.75
	Group II	24.12 ± 1.24	24.12 ± 1.24	24.12 ± 1.24	24.12 ± 1.24	24.12 ± 1.24	24.12 ± 1.24
	Group III	24.16 ± 1.45	21.12 ± 1.05 ^{NS}	20.10 ± 1.25	18.10 ± 1.12*	16.12 ± 1.00*	14.14 ± 1.15*
	Group IV	24.18 ± 1.22	23.10 ± 0.75 ^{NS}	22.16 ± 1.15 ^{NS}	21.16 ± 1.67 ^{NS}	21.19 ± 1.54 ^{NS}	20.16 ± 1.62*
Total Lipids (mg/mg wet wt. of tissue)	Group I	36.12 ± 1.20	36.12 ± 1.20	36.12 ± 1.20	36.12 ± 1.20	36.12 ± 1.20	36.12 ± 1.20
	Group II	36.15 ± 1.03	36.15 ± 1.03	36.15 ± 1.03	36.15 ± 1.03	36.15 ± 1.03	36.15 ± 1.03
	Group III	36.10 ± 2.02	34.14 ± 1.02 ^{NS}	32.16 ± 1.25 ^{NS}	30.00 ± 1.02 ^{NS}	26.18 ± 1.82*	23.12 ± 0.78*
	Group IV	36.25 ± 1.05	35.15 ± 1.00 ^{NS}	35.12 ± 1.00 ^{NS}	34.22 ± 0.50 ^{NS}	34.19 ± 1.05 ^{NS}	33.00 ± 0.01 ^{NS}

4.DISCUSSION

Proteins are involved in major physiological events therefore the assessment of the protein content can be considered as a diagnostic tool to determine the physiological phases of organism. Proteins are highly sensitive to heavy metal poisoning (Jacobs *et al*., 1977). The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of mercury chloride of the lower exposure period (Shakoori*et al*., 1994) due to their utilization in the formation of mucoproteins which are eliminated in the form of mucus. Further, direct and /or indirect utilization of proteins and lipids for energy need was also reported (Nagai and Ikeda., 1971). Liver being the centre for various metabolisms is also rich in proteins. Protein are important organic substances required in tissue building and repair. Under extreme stress conditions, protein supplies energy in metabolic pathway and biochemical reactions (Winer1971). Torreblanca *et al*., 1991 observed decrease in protein content in the fish *Fundulus rolitus* stated that the aquatic inhabitants exposed to toxic conditions utilized protein as energy source. A number of workers have reported depletion in the protein level in different tissues/organs of experimental animals under the stress of various metals, pesticides, chemicals etc, (Mastan 2008). The depletion of protein also suggests increased proteolysis and possible utilization of the product of their degradation for metabolic processes (Bhilave *et al*., 2008). The depletion of total protein content may be due to break down of protein into free amino acid under the effect of mercury chloride at the lower exposure period (Shakoori *et al*., 1994). Hence high activity of protease, a lysosomal enzyme, in the organs caused damage by mercury to lysosomes (Martin Deva Prasath and Arivoli., 2008). Elevated protease activity induced proteolysis may be the increase in free amino acid pool due to increased proteolysis would act as an osmotic and ionic effectors to bring the electrostatic equilibrium between the external medium (Schmidt Nielson, 1975; Jurss 1980).

The decreased glycogen concentration in the test fish could be due to its enhanced utilization as an immediate source to meet the energy demand under heavy metal stress. Fish are largely used in evaluation in the quality of aquatic systems and some of their physiologic changes can be considered as biologic markers of environmental pollution (Dautremepuits *et al*., 2004). Depleted glycogen level under heavy metal stress was reported in common carp *Cyprinus carpio* (Vinodhini and Ramaswamy, 2008) and this supports the reduction of hepatic synthesis of detoxifying enzymes requires high energy levels (Begum and Vijayaraghavan. 1995, Hori *et al*., 2006). Reduction of carbohydrate rates in the reproduction and other tissues indicated the possibility of active glycogenolysis. Tissue acidosis due to reduced oxygen transport must have also favoured the process of glycogenolysis in tissues (Senthilkumar *et al*., 2007). In the present study the depletion of glycogen in the liver indicate rearrangement of this energy store as reported by Nordgarden *et al*., 2002. Alterations in the chemical composition of the aquatic environments affect the behavior and biochemical system of fish (Radhaiah, *et al*., 1987) Changes in enzyme activities and alterations in glycogen, glucose and lipid metabolism in fish due to lindane intoxication have also been studied by Ferrando and Moliner (1991), Gopal *et al*., (1993). The carbohydrate source is stored as a reserve fuel in the liver and muscle tissues of fish for endogenous derivation of energy during acute and chronic stress (Bonga 1997). Prolonged stress after metal toxicity exerts weakness and hypoxic condition with the inability of hepatocytes to propagate the regular cellular metabolism (Heath, 1995). The decreased glycogen content in fish *C. batrachus* which was also observed during the present study, alters the enzymes of carbohydrate metabolism and might be utilized in the formation of glycoproteins and lipids (Levesque *et al*, 2002). It is presumed that the biochemical changes in fresh water fishes are mostly to cope with usual environmental stressors, including hypoxia (Raja and Kulkarni 2008). It was shown that glucose levels in fish were affected by many stress factors, including heavy metals (Canli, 1995). Glucose content in the two tissues under study was found to decrease continuously throughout the exposure period. Depletion in glucose in the present study may be due to its rapid utilization to meet the demands under toxic manifestation (Bhilave *et al*., 2008). In conclusion the disturbance in the carbohydrate metabolism was considered as one of the most outstanding biological lesions due to the action of pesticide and glucose utilization to meet excess energy demand as well as the condition and response of the test organism to the pesticide, the degree of retention and the rate of excretion.

In relation to the total lipid in this study it was found that there was significant decrease of lipid in the pesticide intoxicated groups as comparted to control. Lipids are also the storage form

of energy like glycogen. There is a decrease in the order of lipid level in liver in the exposed fish was noticed. Earlier studies have also shown that lipid and protein concentration of liver depleted in fishes exposed to heavy metal chromium (Ambrose *et al*., 1994 and Deepali Sexena and MadhuTripathi 2007), Copper (Senthilkumar *et al*., 2007), Mercury chloride (Martin Deva Prasath and Arivoli 2008) and Cadmium (Levesque *et al*., 2002, Fabien Pierron*et al*., 2007). According to them decrease in tissue lipid might be partly due to their utilization with the formation of lipoproteins which are important cellular constituents of cell membranes and cell organelles present in cytoplasm (Harper *et al*., 1977). Lipids are also the storage form of energy like glycogen. The lipid levels also decreased in the tissues of the fish supplemented with Vitamin – C in the present study may be attributed to its utilization in cell repair and tissue organization. Lipids act as reversed depot of energy from where the energy is supplied as and when required (Katti and Sathyanesan, 1983; Bhilave *et al*., 2008). In stress condition induced by pesticides, the lipid content depleted to meet the energy demands. From this study it is clear that the ascorbic acid supplementation enhances fish tolerance to environmental stress and reduces malathion toxicity in liver of the fish *Clarias batrachus*.

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