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Effect of Some Pesticides on the Antioxidant Enzymes and Lipid Peroxidation in Carp Tissues

By

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ABSTRACT

A Pilot study was carried out to measure specific activity and distribution of antioxidant enzymes; catalase (C-ase) and superoxide dismutase (SOD) and lipid peroxidation (LP) in brain, liver, kidney, gill and muscle of common carp (Cyprinus carpio L.). The highest level of catalase was found in liver (466.6 units/gm wet tissue) followed by kidney. The activity of SOD in liver was two-fold that in kidney. And the highest level of LP was observed in brain.

The in vivo effects of half LC_{50} and LC_{50} of pyrazophos, glyphosate, fenvalerate and copper sulfate after 96 hours on C-ase, SOD and LP of fish were studied. The greatest induction of liver C-ase was noticed by glyphosate, while the least was by $CuSO_4$. Different pesticides have different effects on SOD activity, oscillating between activition and inhibition. Brain, kidney and liver were chosen to evaluate the effect of pesticides on lipid peroxidation as a food quality. Liver was the most vulnerable to this harmful phenomenon that was highly induced.

INTRODUCTION

Many pesticides are extremely toxic to fish in the environment (Pimental, 1971). Moreover, aquatic biota consumed as food stuff represent a source of human exposure to toxic xenobiotics. The bioconcentration of toxicants by aquatic organisms is considered to be the most important problem among the chronic effects of pesticides to non-target organisms.

Lipid peroxidation has been broadly defined by Tappel (1973) as the oxidative deterioration of polyunsaturated lipid. Lipid peroxidation has been shown to be an exceedingly damaging biological process. The most general defense of aerobic organisms against lipid peroxidation lies in; the structural characteristics

of cell membranes (Rouser et al., 1968) and in antioxidant enzymes include SOD (Mc Cord and Fridovich, 1969) and catalase (Beers and sizer, 1952).

The objective of this work was to study the specific activity of catalase and superoxide dismutase and lipid peroxidation in different tissues of common carp. Then to evaluate the effect of LC_{25} and LC_{50} of four common pesticides (Pyrazophos, glyphosate, fenvalerate and Cu SO_4) on these enzymes.

MATERIALS AND METHODS

1 . Chemicals:

Four pesticides were used:- Pyrazophos, 30 % EC, Hoechst company, west Germany; Glyphosate, 48 % EC, Monsanto Company, USA; Fenvalerate, 20 % EC, Sumitomo chemical company, Japan. And Copper Sulfate (Chemical Pure).

2 . Tested animals:-

The common carp (Cyprinus carpio L.)was collected from saft Khalid farm in Etay AL-Barood. The fish was kept in large tanks with aerated tap water and acclimated to laboratory conditions for two weeks before the experiment began.

3 . Biochemical Studies:-

a . Distribution of C-ase, SOD and LP in different tissues of fish:-

A pilot study was carried out to measure the level of C-ase, SOD and LP in brain, liver, kidney, gill and muscle.

b . In vivo effects of half LC_{50} and LC_{50} of tested pesticides on C-ase, SOD and LP of common carp:-

The experiment was conducted using nine groups (ten fishes each). Eight groups were treated with half LC_{50} or LC_{50} values of pyrazophos, glyphosate, fenvalerate and copper sulfate (EL-Gendy et al. 1990), and the ninth group was used as a control. Three fish each from control and treatment were killed after 96 hours. Then, brain, liver, kidney and muscle were removed and homogenized in 10 volumes (W/V) of physiological saline solution. The homogenates were then centrifuged at 6,000 x g for 30 minutes. The supernatant was used for each assay.

Catalase assay:- The enzyme activity was estimated by the method of Beers and sizer (1952). The enzyme activity is given in Bergmeyer units (BU) per gram wet tissue. A unit is the amount of enzyme which liberates half the peroxide oxygen from a hydrogen peroxide solution of any concentration in 100 seconds at 25 °C.

Superoxide dismutase assay:- SOD activity was measured by the adrenochrome method of Misra and Fridovich (1972) and Hien, et al. (1974). The essence of the method is that adrenaline transforms spontaneously to adrenochrome in the presence of air at pH 10.2. This spontaneous oxidation of adrenaline is inhibited to an extent depending on the amount of SOD. Activity of SOD is calculated as units per gram wet tissue. A unit of SOD can be regarded as that amount of enzyme which causes a 50 % inhibition in the extinction change in one minute compared to the control.

Lipid peroxidation assay:- Determination of lipid peroxidation is carried out according to the method of placer, et al. (1966) and Nair and Turner (1984), using thiobarbituric acid. Lipid peroxidation is expressed as n moles of malondialdhyde(MDA) per ml.

All the data were expressed as mean ± SD and were statistically analyzed by student's T-test with $P<0.05$ as the level of significance.

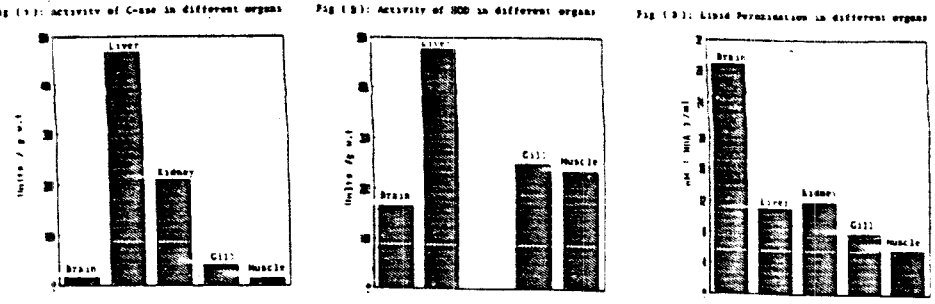
RESULTS AND DISCUSSION

Catalase :-

Catalase is the first line of defence against H_2O_2, levels of catalase activity in different organs of common carp were repersented graphically in fig 1. The highest level of enzeme activity was found in liver (468.6 units/g w t) followed by kidney, while the least was in brain and muscle. Our results agree with Matkovics, et al. (1977) who found that the liver, spleen, RBC and kidney possessed the highest catalase activity in some important experimental animals, while the brain contained the least activity. Therefore, the liver and kidney were picked for further studies [illegible] the in vivo effects of [illegible] and [illegible] of paratophos, glyphosate, [illegible] and $CuSO_4$ on catalase activity in fish. The obtained results indicated that the liver and kidney catalase activities in treated fish were with higher [illegible] table [illegible]. This induction was highly significant in liver. The greatest activation was [illegible] glyphosate and the least was by $CuSO_4$. Also it was noticed that the effect on catalase activity was dose dependent.

Superoxide dismutase :-

Preliminary experiments showed that liver contained almost twice as much as the SOD activity of brain, gill and muscle, while kidney showed no demonstrable activity (fig. 2). This data was parallel to that by Hien, et al. (1975) and Matkovics, et al. (1977).



Liver was selected to estimate the effect of tested pesticides on fish SOD activity. It is clear, as shown in table 1 , that the different pesticides have different effects on SOD activity, oscillating between activition and inhibition. Inhibition was noticed at LC_{50} of glyphosate (54.54 %) and half LC_{50} of Cu SO_4 (45.1 %). No change in the activity of liver SOD was produced by LC_{50} of Cu SO_4. Activition was caused by all other treatments.The greatest activition was noticed by half LC_{50} and LC_{50} of pyrazophos and the least activition was by LC_{50} of fenvalerate.

Lipid peroxidation:-

Lipid peroxidation has been broadly defined as the oxidative deterioration of polyunsaturated lipid. Lipid peroxidation, as an undesirable food quality, was demonstrated at its peak in brain followed by liver and kidney and at its least level in gill and muscle (fig. 3). It should be mentioned that fish tissues contain a considerable quantity of lipid, mainly phospholipids which are readily decomposable. It is well known that the amount of polyunsaturated fatty acids is high in brain, kidney and liver. This is parallel to the level of lipid peroxidation.

Brain, kidney and liver were chosen for further studies to compare the effect of pesticides on lipid peroxidation. Liver was shown to be the most vulnerable to this harmful activition, less activition was noticed in the kidney, and the least activition was demonstrated in brain (table 1).

we can conclude that pyrazophos, glyphosate, fenvalerate and Cu SO_4 induced significantly the activity of superoxide metabolism enzymes (C-ase and SOD) and lipid peroxidation . The role of enzymes systems that can metabolize lipid peroxides or inhibit their formation is very important. SOD is an enzyme that acts as a primary biological protector, which defends cells from the damaging effect of superoxide radicals ($\bar{O}_2^{\cdot}$) by dismuatation to hydrogen peroxide and oxygen (Mc Cord and Fidovich, 1969). Catalase, eliminates hydrogen peroxide (Wdzieczak, et al. 1981, 1982 and Gabryelak, et al. 1983). An increase of the activity of these enzymes can represent an undesirable food quality in fish. Sublethal levels of pesticides seem to be harmful from this point of view.

Table (1): In vivo effects of LC_{25} and LC_{50} of tested pesticides on catalase, superoxide dismutase and lipid peroxidation in different organs of common carp (Cyprinus Carpio L.) after 96 hr.

		S.A (mean ± S.D)								
			Pyrazophos		Glyphosate		Fenvalerate		$CuSO_4$	
Parameters	Organs	Control	½ LC_{50}	LC_{50}	½ LC_{50}	LC_{50}	½ LC_{50}	LC_{50}	½ LC_{50}	LC_{50}
C-ase	liver	468.6 ± 33.2	764 ± 15**	2002 ± 123**	1734 ± 0.0**	2185 ± 112**	834.2 ± 118.8**	956.2 ± 106.3**	781 ± 65.2**	842.8 ± 168.2**
	Ridney	212.5 ± 0.0	224.3 ± 11.8	247.9 ± 35.5	250 ± 10	397.3 ± 27.7**	229.7 ± 0.9	276.5 ± 26.3*	187.4 ± 17.4	230.3 ± 12.5
SOD	liver	500	1562.5	1543.4	806.5	227.3	1000	628.1	274.7	500
lipid Peroxidation	brain	28.9 ± 2.0	32.6 ± 6.1	70 ± 4.4**	85 ± 8**	65 ± 3**	29.5 ± 0.3	31.4 ± 3.6	38.3 ± 2.2*	70.5 ± 5.5**
	Ridney	11.9 ± 1.1	55.9 ± 1.7**	26 ± 1.1**	33.7 ± 2.2**	31.3 ± 1.2**	18.4 ± 1.1**	20.6 ± 1.1**	16.9 ± 0.6**	22.3 ± 4.9
	liver	11.1 ± 0.8	59.7 ± 2.7**	22.3 ± 2.2**	16.8 ± 2.2**	40.7 ± 1.7**	35.3 ± 2.7**	43.4 ± 0.0**	67.4 ± 2.5**	69.3 ± 9.6**

C-ase activity is expressed as (Units / gm wet tissue)
SOD activity is expressed as (Units / ml)
lipid peroxidation is expressed as (n moles malondialdehyde / ml)
* Significantly different from control ($P < 0.05$)
** Highly significant different from control ($P < 0.05$)

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الملخص العربي

تم تقدير مستوى انزيمى الكتاليز والسوبر أوكسيد ديسميوتيز وأيضا عملية أكسدة الدهن فى الأعضاء المختلفه لسمك البورى (الكبد - الكليه - الخياشيم - الخ - العضلات) وقد وجد ان الكبد أكثر الأعضاء نشاط لهذين الأنزيمين . بينما الخ كان اكثر الأعضاء التى يتم فيها عملية اكسدة الدهن نتيجه لارتفاع نسبة الدهن فيه .

كما درس تأثير تعرض الاسماك للتركيز المسبب لموت نصف الافراد ونصف هذا التركيز لمدة ٩٦ ساعه من مبيدات البيرازوفوس - الجليفوسات - الفنتاليرات وكبريتات النحاس على انزيمى الكتاليز والسوبر أوكسيد ديسميوتيز وعملية أكسدة الدهن وقد وجد أن المبيدات المختبره كان لها دور كبير فى زيادة نشاط انزيمى الكتاليز والسوبر اوكسيد ديسميوتيز بالاضافه لزيادة عملية اكسدة الدهن .