THE EFFECTS OF SHORT-TERM EXPOSURE TO ORGANOPHOSPHATE INSECTICIDES ON SOME BIOCHEMICAL PARAMETERS IN COMMON CARP (*Cyprinus carpio L.*)

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ABSTRACT

The aim of the study was to assess the effects of short-term exposure to organophosphate insecticides on some blood biochemical parameters on common carp (Cyprinus carpio L.) harvested from the Tundzha River, Bulgaria. In treated group there were significant (P<0,05) alterations found in the activities of aspartate aminotransferase (AST), creatine phosphokinase (CPK), lactatdehydrogenase (LDH) and urea (P<0,001) as well as in the serum concentrations of alanine aminotransferase (ALT) and amylase. The creatinine concentrations in the exposed fish were statistically (P<0,05) lower than the controls. The differences between investigated serum biochemical parameters of the treated and control groups were not great (except LDH) and we assume that they could return to normal after a short time.

INTRODUCTION

Pesticide is a substance, or mixture of substance, that are used to control pests such as plant parasitic viruses, bacteria, nematodes, fungi, insects, weeds, rodents, and birds. Pesticides include all materials that are used to prevent, destroy, repel, attract, or reduce pest organism. They find application in different area forestry, landscaping, agriculture and domestic use (Sivaperumal, 2008). Fish are appropriate aquatic vertebrates to be used as environmental bioindicator organisms (Mitev et al., 2013). The pollutants such as organophosphate insecticides (OPs) may damage certain physiological and biochemical processes when they enter into the organs of fishes (Nagaraju and Rathnamma, 2013). Fish are sensitive to water contamination, and pollutants may impair many physiological and biochemical processes when assimilated by fish tissue (Mitev et al., 2014). Organophosphates are esters of phosphoric acid, are classified according to the radicals bound to phosphor particles. Toxicity may vary according to the molecule, pharmaceutical form and the affected animal species. (Sivaperumal, 2008). Contamination of water by insecticides is mainly due to intensive agriculture combined with surface runoff and subsurface drainage, usually within a few weeks after application. In fish, different insecticides can be absorbed through gills, skin or alimentary ducts (Banaee, 2013). It was proposed that temporal variations in biochemical parameters of blood must be considered when these parameters are used to evaluate fish health status (Bani and Vayghan, 2011).

The aim of this work was to study the effects of short-term exposure to organophosphate insecticides on some biochemical indices (AST, ALT, LDH, CPK, amylase, urea, and creatinine) in blood of common carps (*Cyprinus carpio L*.).

MATERIALS AND METHODS

The fish samples were obtained from the Tundzha River near the city Nikolaevo, Bulgaria. The region was chosen because of the agricultural activities of the district. Six samples of carp fishes, were taken twice and transported in a conveying tank, reinforced with oxygen to the Experimental Aquaculture Base at Trakia University, Bulgaria. Blood (2,5-3,0 ml) was drawn from the *vena caudalis* using a needle (18G). After coagulation

obtained serum was immediately separated and it was stored at -20°C until analysis. The concentrations of were determined by a kit ”Chema Diagnostica” on a biochemical analyzer Mindray BS-200.

Statistical analysis was performed using SPSS statistical software (version 19.0; SPSS Inc., Chicago, IL, USA). All results are presented as mean and standard deviation of the mean (Mean ± SD).

RESULTS AND DISCUSSIONS

Studies of biochemical changes in the fishes exposed to insecticides can help to supplied information on the impact of insecticides on fish health. The obtained results are shown in table 1.

Table 1

Blood serum biochemical parameters in common carp before and after short-term exposure to organophosphate insecticides

Parameters	***Groups***	***Mean ± SD***
Urea (mmol/L)	Control	1,48±0,24
	Experimental	1,85±0,05
Creatine (µmol/L)	Control	38,33±11,68
	Experimental	9,00±5,29*
ASAT (U/L)	Control	70,33±8,50
	Experimental	105,67±13,61*
ALAT (U/L)	Control	26,33±7,09
	Experimental	32,00±4,36
Amylase (U/L)	Control	54,00±6,56
	Experimental	67,33±41,43
LDH (U/L)	Control	187,67±24,79
	Experimental	581,67±79,78**
CPK (U/L)	Control	1691,67±170,12
	Experimental	2153,33±62,50*

For a given biochemical parameter: *(P<0,05) and **(P<0,01) indicate significant differences between control and experimental groups.

As with mammals, it has been shown that after functional damage to tissues and organs of fish, some specific cellular enzymes leak into blood plasma where they have been detected (Krajnovic-Ozretic and Ozretic, 1987).

The changes in the enzyme activities of LDH, AST, and ALT have been used for demonstrating tissue damage in fish (Asztalos et al., 1990). Furthermore, hepatocyte injury leads to the release of tissue-specific enzymes into the bloodstream. Transaminases are a group of enzymes that catalyze, the process of biological transamination. The most important and used extensively are alanine- and aspartate aminotransferase (ALT; AST) which are biochemical markers of hepatocellular injury. In fishes, measurement on these enzymes has reduced use. In our study these enzymes were higher than controls, especially AST. Stojkovski (2001) published that in fish’s normal blood aspartate- and alanine aminotransferase activities varied between 15-165 and 3-42 UI/L, respectively. In our research there was a significant (P<0,05) increase in AST activity (105,67±13,61 U/L) compared to controls (70,33±8,50 U/L) while the enhancement in ALT activity was not significantly (32,00±4,36 U/L compared to controls 26,33±7,09 U/L). The increasing activities of aminotransferases could be due to raising protein synthesis or another reason could be that the toxins damage the cell membranes and evoke release of enzymes into

the blood stream. Raised levels of blood fishes ALT and AST activities from polluted water was also observed by Yousafzai et al., (2008).

Elevated lactate dehydrogenase (LDH) activities are a marker for tissue damage in fish and serve as a good diagnostic tool in toxicology (Sivaperumal, 2008). LDH mediates the inter-conversion of lactate and pyruvate and present in numerous tissues, cytoplasm, enzymes and it is a marker of tissue damage. Injured organs and tissues release LDH into the haemolymph, which raises the level of this enzyme, and greater the degree of tissue damage greater is the release of LDH. (Sivaperumal, 2008). In our study LDH activity was significantly (P<0,001) higher (581,67±79,78 U/L) when compared to control (187,67±24,79 U/L) which indicate cellular damages. The increase of liver specific enzymes (AST, ALT, and LDH) indicate a possible liver damages in fishes.

Creatine phosphikinase (CPK) catalyses the reversible exchange of high-energy phosphate bonds between phosphocreatine (PCr) and ADP (adenosine diphosphate), regenerating ATP (adenosine triphosphate) from ADP produced during muscle contractions. CPK are important for supporting cellular energy homeostasis. The levels of CPK are often found to be elevated in organophosphorus poisoning. I was examined by Vanneste and Lison, (1993) that several studies on rat liver and fresh-water snails show that there is a connection between OP poisoning and CPK levels. Hassan and Madboly (2013) have confirmed high degree of correlation between CPK value and severity of OP poisoning. Other authors like Bhattacharyya et al., (2011) recommends CPK can use as an alternative marker of cholinesterase levels in blood to assess the severity of organophosphorus poisoning. The referent ranges for CPK activity are between 191-1312 U/L (Stojkovski, 2001). In our investigation in both controls and treatment carps the CPK values are higher than normal. The measurement show that experimental fishes indicated statistically higher (P<0,05) levels 2153,33±62,50 U/l compared to baselines-1691,67±170,12 U/l. This enhancement could be attributed to the muscle fiber necrosis and needs to be confirmed by muscle biopsy.

The dynamics in the concentration of any biochemical parameters (AST, ALT, CPK) in our study were in agreement with data reported by Velisek et al., (2011) who writes about effect of the toxicity of pyrethroid pesticides on different types of fish.

Sumathi et al., (2014) offers serum amylase and CPK can be used as an additional prognostic indicators with plasma cholinesterase levels in cases with OP poisoning. Serum amylase could be considered as a better predictor of severity followed by CPK. According to Sumathi et al., (2014) hyperamylasemia in cases of OP poisoning, may be due to the fact that acute pancreatitis is caused by excessive cholinergic stimulation of pancreas by OP compounds. Ahmed et al., (2009) found that elevation of serum amylase is common in patients with pesticide poisoning. The result of our study confirm those reports. Mean values of amylase in treated carps was higher (67,33±41,43 U/L) compared to controls 54,00±6,56 U/L. However, the values remained within the normal range for this species according to Stojkovski, (2001) up to 70 U/L.

Piscine kidneys contribute little to the excretion of nitrogenous wastes also interpretation of the levels of urea nitrogen and creatinine may not be useful in the estimation of renal function and diseases (Thrall et al., 2012). Creatinine is formed from creatine and secreted by fishy kidneys. The normal ranges are between 44 µmol/L and 177 µmol/L in which our baseline values are close to the lowest of them-38 µmol/L. Urea is derived mainly from degradation of purines via uric acid. Most fish produced small quantity of urea and the gills are the major organ of urea excretion for some of them. Also, enlargement in the values of the urea may be more indicative of branchial epithelial disease than of renal disease. The normal urea concentrations of freshwater teleost is less than 3,57 mmol/L (Thrall et al., 2012). In our investigation the levels of controls and treatment carps are under this value. Likewise, according to Nicula et al. (2010) the

concentration of urea in cyprinids are 2,02 mmol/L which is very close to the levels found by us. It was reported that exist data about more large limits for blood urea on different species of fish: from 0,06 mmol/L to 2,6 mmol/L. Our results indicated than the urea concentration remained in both of groups in reference ranges for this species.

CONCLUSION

The biochemical profiles determined in the present study suggest that homeostasis of common carps were slightly changed after short-term exposure to organophosphate insecticides. However, the differences are not great, and we presume that they were reversible.

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