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Pesticides induce fatty degeneration in liver of Cyprinus carpio (Linnaeus 1758) after acute exposure

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Abstract. In the present study, results are presented regarding the negative effects of different concentrations of three classes of pesticides - an insecticide, herbicide and fungicide on one relatively uncommon, but reliable histochemical biomarker in the field of fish toxicology – lipid accumulation in the liver of common carp under laboratory conditions.

Key words: fish, liver, pesticides, contamination, biomarkers.

Introduction

As stated by Kılıç (2020), Nayak et al. (2023) and Rohani (2023) freshwater resources are vital due to their use for human consumption, different production activities, agriculture irrigation, and biodiversity support. According to Kılıç (2020) factors that cause negative effects on aquatic ecosystems include various chemical pollutants, industrial wastes, synthetic detergents, and pesticides, etc. In this regard, pesticides pollution in the aquatic ecosystems affect the organisms, such as fish, directly by resulting in different changes causing mortality or destructing their available food resources (Abdelmeguid et al., 2002; Da Cunã et al., 2011). In addition, according to Rohani (2023) the indirect negative effects of pesticides could be related to disruption of growth and development rates of fish, as well as their survival rate.

The application of biomarkers for monitoring, both environmental quality and the health status of the aquatic organisms, inhabiting polluted ecosystems, has received increasing attention in recent years (Kroon et al., 2017; Marinsek et al., 2022; Nagarani et al., 2023). Therefore, the assessment of water quality should be based not only on the physicochemical characteristics of the environment, determined by chemical analyses (Cave et al., 2011; Kuklina et al., 2013; Olsvik et al., 2019), but also on studies related to histopatological alterations in the fish organism, which could be applied as potential biomarkers for the effects of water contamination. Considering one of the main liver functions related to biotransformation, this organ is the major target for xenobiotics (Au, 2004; Da Cunã et al., 2011). As stated by Cave et al. (2011) and Olsvik et al. (2019) abnormal lipid accumulation in the liver is among the most common pathological liver responses, resulting from chemical exposure. The liver is thus, considered to be an interesting model in the ecotoxicological studies, which serves as a target organ for the interactions between environmental factors and hepatic functions (Bruslé & Anadon, 1984; Faccioli et al., 2014). Willebrords et al. (2015) added that the number and size of lipid droplets, typically present in the hepatocyte cytoplasm, are well-established histochemical biomarkers of a fatty liver. Furthermore, elevated free fatty acids in the liver cause the synthesis of lipotoxic metabolites, leading to oxidative stress, mitochondrial dysfunction, and thereby development of liver inflammation (Buzzetti et al., 2016; Zheng et al., 2021). In this regard, hepatotoxicity is a key part in risk assessment programs and a common toxic characteristic of many environmental contaminants, including pesticides (Armstrong & Guo, 2019).

The main aim of the present study is to determine and compare the expression of histochemical changes in the liver of the bioindicator species common carp (*Cyprinus carpio* Linnaeus, 1758).

Materials and Methods

Pesticide concentrations

We used three groups of pesticides – an insecticide, herbicide and fungicide with their respective active ingredients – pirimiphos-methyl, 2,4-dichlorophenoxyacetic acid (2,4-D) and propamocarb hydrochloride. The tested concentrations were prepared after we diluted LC_{50} of each used chemical and they were as follows: 10 µg/L and 60 µg/L ot the tested insecticide; 50 µg/L and 100 µg/L of the tested herbicide; and 40 µg/L and 80 µg/L of the tested fungicide, respectively as explained in our previous experiment (Kovacheva et al., 2022).

Test organism

According to Yancheva et al. (2022), common carp is one of the most commonly reared fish in aquaculture, which is a key feature for the selection of bioindicators in laboratory and field experiments. Common carps, with normal morphology and no observed lesions, were provided by the Institute of Fisheries and Aquaculture (Plovdiv, Bulgaria). According to de Moura et al. (2017) fish were placed in 100-L glass aquaria for two weeks for acclimatization. Thereafter, the fish were divided randomly into eight groups (n = 15) for the purposes of the 96 hours' experiment, which was performed in static conditions. An aquarium (n = 15) with no added chemical served as a control group. According to APHA (2005), the physicochemical characteristics of the water - conductivity, dissolved oxygen, pH, and temperature were measured during the experiment with a multi-portable meter (Multi-Line® Multi 3510 IDS, WTW-Xylem Analytics, Weilheim, Germany) on a daily basis.

Histochemical analysis and assessment

Fish dissection was carried out according to Directive 2010/63/EU, regarding the protection of animals used for scientific purposes. Liver cryosections (6 µm) from the test individuals were prepared with a cryostat (Leica CM 1520, Wetzlar, Germany). They were stained with Sudan Black B kit (SkyTek Laboratories Inc., Logan, USA) for the detection of lipid accumulation in the exposed hepatocytes (Sheehan & Storey, 1947; Bronner, 1975). The histochemical changes in the liver were presented according to a semi-quantitative scale described by Mishra & Mohanty (2008), which we slightly modified. The liver cryosections were observed with a light microscope (Leica DM 2000 LED, Leica Microsystems, Wetzlar, Germany), connected to a microscope camera (Leica MC170 HD, Leica Microsystems, Wetzlar, Germany). The lipid accumulation was assessed as follows: (0) – negative reaction to histochemical staining; (1) – very weak histochemical reaction; (2) – weak histochemical reaction; (3) – moderate histochemical staining reaction with intense blue to black staining; (4) – severe histochemical reaction in the hepatocytes with intense blue to black staining.

Statistical analysis and assessment

The results from all conducted histochemical analysis were expressed as mean ± standard deviation (SD). GraphPad Prism 7 for Windows (USA) were applied for the statistical analyses in the present study. T-test was used to study the significant difference between the treated and control fish groups. The levels of statistical significance were set at $p < 0.05$.

Results and Discussion

The physicochemical parameters of the water stayed relatively constant during the acute experiment. No significant changes between the control and the tested aquaria were observed, therefore they will not be further discussed as previously reported ($p > 0.05$) (Kovacheva et al., 2022).

Based on the obtained results from the conducted histochemical study, we found a weak positive reaction of the histochemical staining in the liver of the control individuals (Table 1). The degree of lipid accumulation in the cytoplasm of the control and treated hepatocytes is presented as follows in Table 1 and Figure 1.

After the exposure to the test insecticide, we observed an increase in the lipid droplets in the hepatocytes at the lower concentration, while we found a tendency towards a decrease in the amount of lipids with an increase in the pirimiphos-methyl concentration. In contrast to the insecticide exposure, we established an increase in the degree of histochemical staining in the hepatocytes of common carp after the exposure to the test herbicide and fungicide.

The obtained results of the Sudan Black B staining method showed a higher degree of lipid accumulation due to propamocarb hydrochloride toxicity compared to 2,4-D exposure. We found a weak positive histochemical reaction after the both applied 2,4-D concentrations. Moreover, the observed intensity of the Sudan Black B staining method was determined as moderate after the acute propamocarb hydrochloride treatment.

In the present study we confirmed that the histochemical methods related to changes in the lipid content in fish hepatocytes can be used as biomarkers for liver damage after an acute pesticide exposure as explained by Gaber et al. (2013). Together with other histopathological methods applied in similar experiments to evaluate changes in the organ, the histochemical alterations are considered a fast, easy and relatively low-cost to perform biomarker for the evaluation of the negative effect degree of pesticide exposure.

According to Liu et al. (2017) and Shah & Parveen (2022) the fish liver hepatocytes are involved in a number of processes related to conversion of glucose to glycogen, lipid control, and aminoacid deamination. We agree with Weber et al. (2020) and Shah & Parveen (2022) that the hepatocyte structural damage in reaction to pesticides may cause liver dysfunction. From our perspective, an interesting result, which we obtained after the insecticide treatment with the pirimiphos-methyl is that we found a significant increase in the lipid content in the hepatocytes only at the lower applied concentration. We consider that this result could be explained with other histopathological changes in the fish liver, such as necrotic changes in the liver cells, which we also determined (unpublished results). In contrast, after the other pesticide treatments we found an increase in the amount of lipid droplets in the cell cytoplasm in parallel with the increasing pesticide concentrations. Similar results related to lipid accumulation were established by Xu et al. (2012) in Prusian carp (*Carassius gibelio* Bloch, 1782) due to 30 days' exposure to trichlorfon and Olsvik et al. (2019) in Atlantic salmon (*Salmo salar* Linnaeus, 1758) hepatocytes due to 48 hours' exposure to chlorpyrifos. According to Qi et al. (2023) pesticide exposure could also induce fatty acid synthesis. As the authors explained, the accumulation of acetyl-CoA increases the production of malonyl-CoA, which is an essential precursor for propanoate metabolism and lipid synthesis. Overall, we found the highest degree of fatty degeneration after the fungicide exposure, which is also an indicator of the higher toxicity of this pesticide compared to the other applied chemicals.

Table 1. Intensity of Sudan Black B staining in common carp's liver after acute pesticide exposure (average results for 15 individuals per treated group).

Test pesticide	pirimiphos-methyl			2,4 - D			propamocarb hydrochloride		
Concentration	control	10 μg/L	60 μg/L	control	50 μg/L	100 μg/L	control	40 μg/L	80 μg/L
Intensity of Sudan Black B staining	1	4*	1	1	2	2	1	3*	3*

(0) – negative reaction to histochemical staining; (1) – very weak histochemical reaction; (2) – weak histochemical reaction; (3) – moderate histochemical staining reaction with intense blue to black staining; (4) – severe histochemical reaction in the hepatocytes with intense blue to black staining; * - statistical significant differences between the treated and control group.

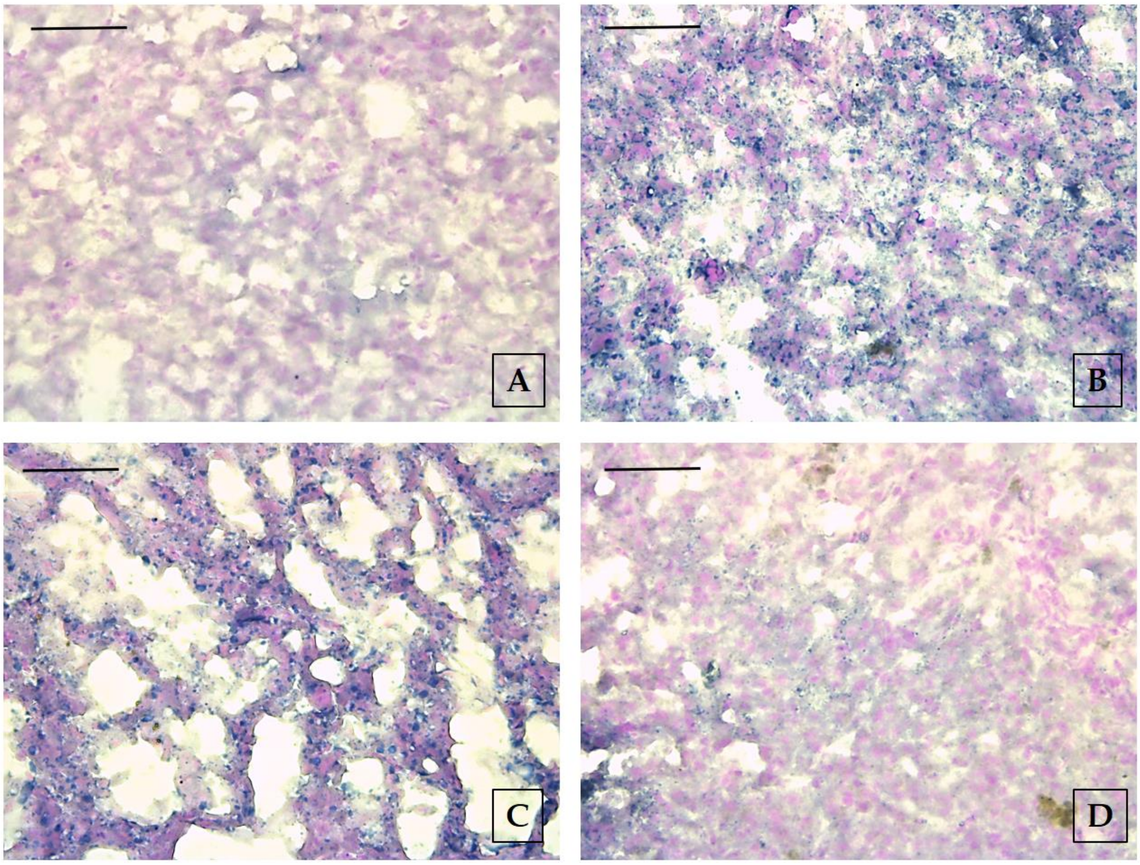

Figure 1. Intensity of lipid accumulation in common carp hepatocytes, Sudan Black B staining, x400: A – control group; B – 10 μg/L pirimiphos-methyl; C – 80 μg/L propamocarb hydrochloride; D – 50 μg/L 2,4 – D.

Conclusions

In a sum, we can conclude that after the 96 hours' exposure to the experimental pesticides, the fungicide had a more significant negative result on the histochemical biomarker in the liver of common carp. The highest degree of fatty degeneration was more pronounced after the fungicide toxicity compared to the insecticide and herbicide. Therefore, we suggest that additional experiments should be carried out with this particular pesticide in order to further study and prevent the negative effects on the physiology of fish.

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