

Toxic effects of permethrin on *Pseudorasbora parva*

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Abstract

The present study deals with acute toxicity and hematological, histopathological and genotoxic effects of permethrin, a synthetic pyrethroid, on *Pseudorasbora parva*. Acute toxicity of 96-hrs LC₅₀ value was found to be 88.25 (84.60-92.63) µg l⁻¹. Sublethal dose was taken as 8.82 µg l⁻¹ (the 1/10 of the LC₅₀ value) in bio-experiments. Micronucleus changes in blood erythrocyte and hemotocyte level in *P. parva* exposed to sublethal concentration were investigated. Difference between nucleolus abnormalities in the experimental and control group was found to be statistically significant (t-test). Micronucleus frequency was found to be 8.26. The hematocrit level in control and experimental groups were found to be 24.43% and 14.673%. No pathological symptoms were observed in the muscle of *P. parva* exposed to sublethal dose for 96 hrs. Pathological symptoms observed after 96 hrs from permethrin administration in other organs were: Fusion, Telangiectasis, epithelial lifting and hyperemia in gills; hydropic degeneration, lipid degeneration and passive hyperemia in liver and enlargement of cavum glomeruli and Bowman space in kidneys and hemorrhage, edema and hyperemia in brain, respectively.

Key words

Pseudorasbora parva, Permethrin, Genotoxicity, Micronucleus test, LC50 value

Introduction

Permethrin [(3-fenoxypheyl) methyl 3-(2,2-dichloroethylen)-2, 2-dimethycyclopropan carboxylate] is a pyrethroid group insecticide. It is largely used in agriculture animal breeding and industry for controlling pest and insect infestation. It is highly effective in aqueous medium (USEPA, 2006). It poses a potential danger to the aquatic world and human health due to high toxicity (Lloyd, 1992). Although it has low level toxicity on birds and mammals (Abernathy and Casida, 1973) it shows toxic effect on fish (Sellami *et al.*, 2014). The effect of synthetic pyrethroids on fish can manifest itself in various ways which range from direct mortality to adverse effects on spawning yield and breeding, which may cause serious depletion in the fish population. Fingerlings are more adversely effected than adults since they are much more prone to environmental effects (Toros and Maden, 1991). Insecticides cause behavioral disorders and negative effects on hematological parameters of fish. Accumulation of these compounds in

living organisms result in toxic, genotoxic or carcinogenic effect (Pandey *et al.*, 2005; Khan *et al.*, 2006; Diran *et al.*, 2006; Zhang *et al.*, 2010).

Acute toxicity of permethrin (LC₅₀) on various fish species has been reported by several authors (Bradbury and Coats, 1989; WHO, 1990; Zhang *et al.*, 2010; Parent *et al.*, 2011). In recent years micronucleus test has been used as an indicator of the health of fish. Micronucleus test of permethrin on *Pseudorasbora parva* erythrocytes is meager. However, scale of micronucleus formation rating fish exposed to different genotoxic agents has been reported to increase to a significant extent. (Yılayaz, 2005; Yılayaz, 2008; Ansari *et al.*, 2009; Selvi *et al.*, 2011; Kan *et al.*, 2012). The adverse changes in the environmental factors are observed to cause a decrease in the hematocrit level of fish (Ahmad and Shakoori, 1995; Uçar and Atamanalp, 2009). There are various studies related to the hematocrit levels of different fish species exposed to different contaminants (Başusta and Şen, 2004; Kandemir *et al.*, 2010).

It is a well known fact that the environmental contaminants have a histopathological potential in aqueous media (Uçar and Atamanalp, 2009). The first apparent effect of contaminants on aquatic organisms are either observed at cellular or tissue level. That is why histological analyses are useful for determining tissue changes (Stentiford *et al.*, 2003). Histological analyses have revealed various diseases and disorders in tissues of the fish exposed to various insecticides and herbicides (Koca *et al.*, 2005; Mikula *et al.*, 2008; Sepici Dinçel *et al.*, 2009; Gül *et al.*, 2012). The studies carried out at natural conditions have showed that the toxicity level did not show a significant difference compared to the values observed at laboratory conditions (Phyu *et al.*, 2013). Tanneberger *et al.* (2013) predicted the acute toxicity of the fish from the gill line base essay.

Pseudorasbora parva is a cyprinid fish species originated from East Asia. They are regarded as an invasive type since it exerts pressure on other species as soon as it has reached the numerical advantage (Ekmekçi and Kirankaya, 2006). There are no studies related to the acute toxicity of permethrin upon *P. parva* in the literature.

In the present study, acute toxicity was studied by 96hrs LC₅₀ value. Sublethal dose used throughout the experiments was 1/10th of the 96h LC₅₀ value. Also hematocrit levels, the major indicator of the health of the fish, of *P. parva* individuals exposed to permethrin was determined. The acute toxicological, hematological, histopathological and genotoxic effects of Permethrin upon *P. parva* species were determined by the changes it caused in tissues.

Materials and Methods

The present study was carried out on an invasive species, Striped Moroko (*Pseudorasbora parva* Temminck and Schlegel, 1846) obtained from Mogan Lake. Ten healthy fish, were used in pre-experiment in order to estimate LC50 value, after accommodation to aquarium conditions. The fish were allowed for 4 days in a 2.5 l experimental aquarium in order to adapt to new aquarium conditions. The fish were not fed 48 hrs prior to experiment. The experiments were carried out by the static bio experiment method and terminated after 96th hrs. Average weight and length of the fish used in bio-experiments were 2.94 g and 7.61 ±cm. The experimental data were evaluated by EPA Probit Analysis determining LC₅₀ and 95% reliability limits (USEPA, 1999).

The study was carried out by the use of Permethrin [(3-fenoxypheñile) methyl 3 - (2,2-dichloroethylenile) -2,2-dimethylcyclopropan carboxylate] (C₂₁H₂OC₁₂O₃), a synthetic pyrethroids group insecticide (96.4%). Permethrin was applied to the system after being dissolved in an analytical

grade acetone: The water used was the tab water conditioned for at least 48 hours get rid of chloride.

There were three different groups namely control, acetone control and positive control (EMS: Ethyl methyl sulfonate) were selected in the experiment. The positive control group was tested with EMS since it is a common chemical compound used for micronucleus test. Micronucleus, hemotocyte and histopathological tests were carried by selected sub-lethal concentration of 8.83 µg l⁻¹ previously defined. The experiments, where this sub-lethal concentration was employed, were completed in 96 hrs. Blood samples were taken from the heart of the fish under ice anesthesia by punctation. Heparin was added to blood samples in order to prevent coagulation. The samples were spread on a slide as a thin film. After drying in an oven, the samples prepared were fixed in 95% methanol for 20 min, stained in 5% Giemsa solution for 20 min and washed with de-ionized water. The micronucleus evaluation was made by counting 1000 cells in each sample under light microscope. The data were evaluated by the use of SPSS 16 statistical software with a significance level of 0.05 and statistical t-test.

Blood samples were taken from the fish exposed to sublethal dose by hematocrit pipettes. The tips of the pipettes were sealed and placed into a microhematocrit centrifuge. Hematocrit percentage was determined after the samples were centrifuged at 11500 rpm for 4 min (Blaxhall and Daisley, 1973; Tanyer, 1985).

Tissue samples taken from gills, liver, muscles, kidneys and brain of the fish exposed to sublethal concentration were dissected under stereomicroscope in a physiological solution, and kept in Bouin fixation solution at least for 24 hrs. The fixed samples were washed for 10 days with 70% ethyl alcohol and blocked in paraffin after dehydration process with increasing ethyl alcohol solutions. Permanent preparations were made by subjecting 6-8 µm thick cross sections taken from the blocks to hematoxylen-eosine staining and closing them by entellan. Cross section taken from various regions of tissue samples were examined and photographed under Olympus BX51 light microscope and the micrographs were transferred to digital media for further evaluation.

Results and Discussion

Acute toxicity : The 96-hrs LC₅₀ value of *P. parva* exposed to permethrin was found to be 88.252 µgl⁻¹ (Fig. 1). There was no mortality in control or acetone control groups. The effect of insecticide on fish was manifested irregular swimming patterns, loss of balance, sudden revolutions around their axis and accumulation under the bottom or top of the water, effort to breath from the surface, going down to bottom and

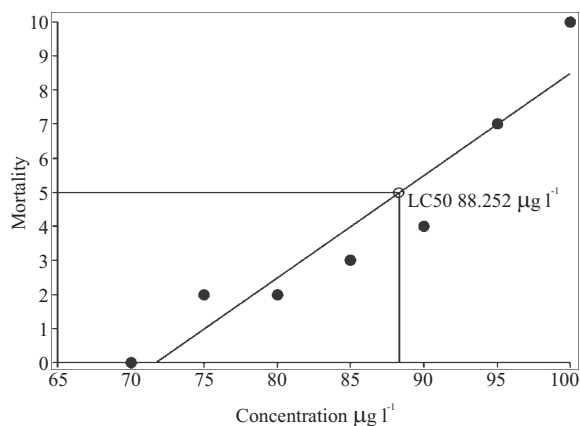


Fig. 1 : Relation between permethrin concentration and mortality in *Pseudorasbora parva*

remaining motionless for long time .

Investigation of acute toxicity of Permethrin on various fish species have showed that 96hrs- LC_{50} values changed between $0.62 \mu\text{g l}^{-1}$ and $56.90 \mu\text{g l}^{-1}$ (Bradbury and Coats, 1989; WHO, 1990). It was reported that Permethrin was much more toxic than the other insecticides (Reza and Gholamreza, 2012). The LC_{50} value determined for *P. parva* was much higher than the values found in other fish species. It is known that fish species show different levels of resistance against toxic compounds. *P. parva* is an invasive species and can easily adapt to different aquatic media.

Micronucleus : Micronucleus test (MN) is useful in determination of genotoxic effects of chemical and physical factors in aquatic media (Melo *et al.*, 2014). The frequency of micronucleus containing erythrocytes show a marked increase in the fish exposed to toxic compounds. In the present study, change of micronucleus in blood samples of *P. parva* species exposed to sub-lethal Permethrin concentration was found to be $88.252 \mu\text{g l}^{-1}$ as 1/10 of 96-hrs LC_{50} value (Table 1). The anomalies observed in micronucleus of the group exposed to permethrin as compared with acetone, EMS and control groups were found to be statistically significant in accordance to 5% t-test. Micronucleus differences in the permethrin experimental group ($t=0.00258$) and the acetone control group ($t=0.002864$) were statistically significant ($p<0.05$).

Ergene *et al.* (1999) found MN frequency of *Clarias lazera* exposed to different concentrations of demethamidophos as 0.18% in control, 1.92% and 3.26% in the experimental groups exposed to 100 ppm and 200 ppm of toxic compound. The corresponding values for *Barbus rajanorum mystaceus* exposed to Parathion methyl were 0.09% for control, 1.35% for 125 ppm and 2.65% for 225

ppm exposure groups (Yılayaz, 2005). It was observed that there is parallelism between this study and the literature data regarding to MN frequencies. The MN frequency of erythrocytes of *P. parva* species exposed to Permethrin were found to be much higher ($8.26/10^3$) than the control group ($0.73/10^3$) (Table 1).

Hematocryte : The hematocryte levels were found to be $24.43\% \pm 5.36$ (17.65-30.77) in control and $14.67\% \pm 0.92$ (13.64-16) in experimental group. The hematocrit level varies according to length and weight, gender seasonal changes and type of fish (Yılayaz, 2008). Also, the environmental pollutants cause to hematocrit level of the fish to decrease (Shahi and Singh, 2011). Synthetic pyrethroids have been reported to significantly decrease in the hematocrit levels of *Ctenopharyngodon idella* (Haider and Rauf, 2014). It was also observed that the exposure of Permethrin causes a significantly decrease at hematocrit levels of *P. parva* in this study.

Histopathology : The morphological and physiological variations observed on the gill, liver, muscle, kidney and brain tissues of *P. parva* are as follows: Red lesions known as a Telangiectasis appeared in some tissues due to enlargement of the capillaries, arterioles and venules; In some tissues, hyperemia was observed as a result of increased or decreased blood flow, regions with hemorrhages also appeared in some tissues.

Table 1 : *Pseudorasbora parva* micronucleus frequencies

Experiment	MN \pm SE (frequency/1000)
Control	0.73 \pm 0.24
Acetone control	1.17 \pm 0.31
EMS control	2.80 \pm 0.67
Permethrin	8.26 \pm 1.36

Table 2 : Histopathological result of various tissues of *Pseudorasbora parva*

Tissue	Pathological result	
Gill	Fusion	+
	Telangiectasis	+
	Epithelial lifting	++
	Hyperemia	++
Liver	Hydropic degeneration	++
	Lipid degeneration	++
	Passive Hyperemia	++
Kidney	Enlargement of Cavum glomeruli and Bowman space	++
	Hemorrhage	++
Brain	Edema Hyperemia	++++

(-): No histopathological result; (+): Histopathological results < 20%; (++): Histopathological results 20-60%

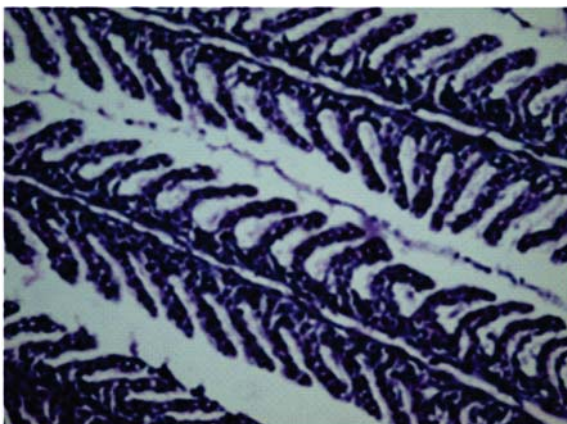


Fig. 2(a) : Gill control group (HE. x20)

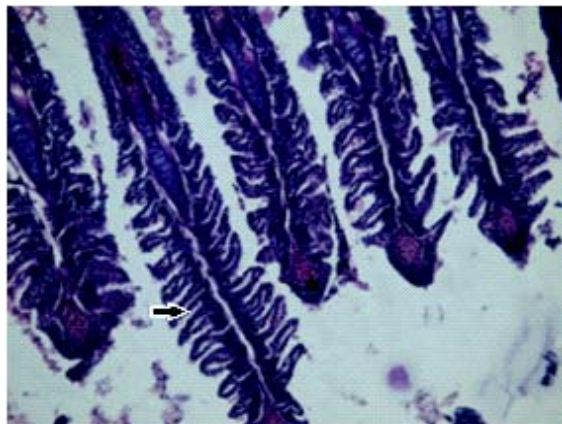


Fig. 2(b) : Epithelial lifting in gills (→)Hyperemia (*) (HE. x20)

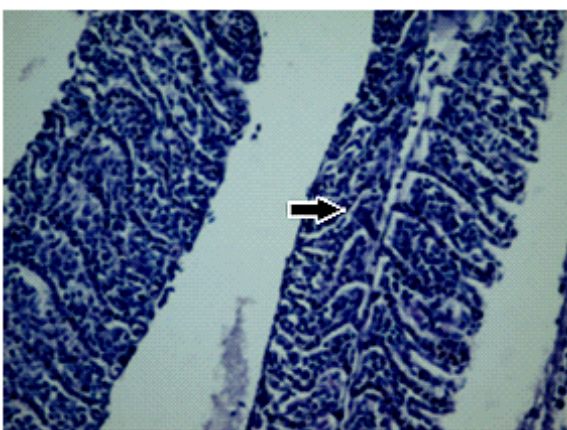


Fig. 2(c) : Fusion in gills (→) (HE. x 20)

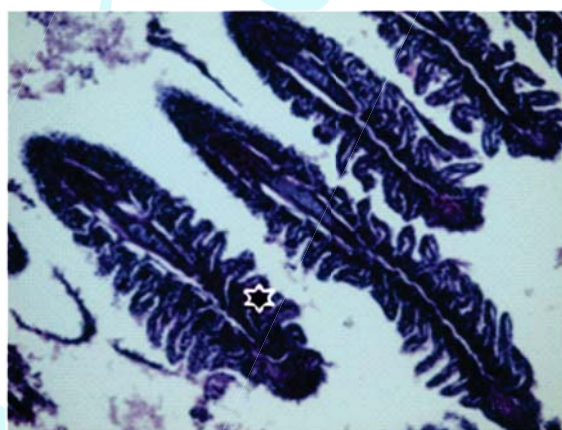


Fig. 2(d) : Telangiectasis in gills (→) (HE. x20)

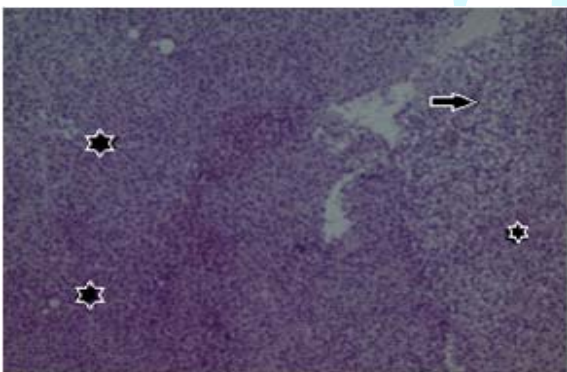


Fig. 3(a) : Passive hyperemia (*) and lipid degeneration in liver (→) (HE. x20)

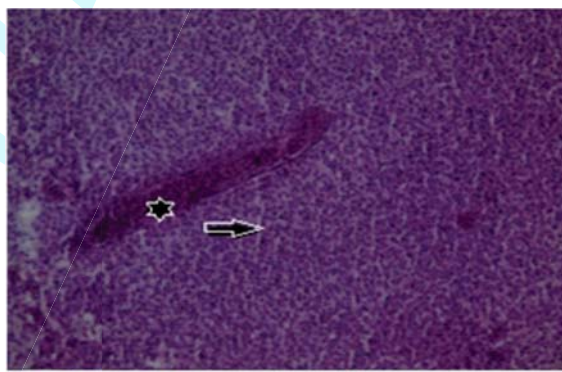


Fig. 3(b) : Passive hyperemia (*) and hydropic degeneration in liver (→) (HE. x20)

It was reported that the pollutants have various degrees of histopathological potentials (Uçar and Atamanalp, 2008). In the present study no histopathological changes were observed in the muscle tissues of *P. parva*. However, fusion, telangiectasis, epithelial lifting and hyperemia were seen in

gills (Figs. 2a,b,c,d); hydropic degeneration, lipid degeneration and passive hyperemia in liver (Figs. 3a,b); enlargement of Cavum glomeruli and Bowman space and hemorrhage in kidneys (Figs. 4a,b,c) and hyperemia and edema in brain (Figs. 5a,b), respectively, as a result of permethrin toxicity.

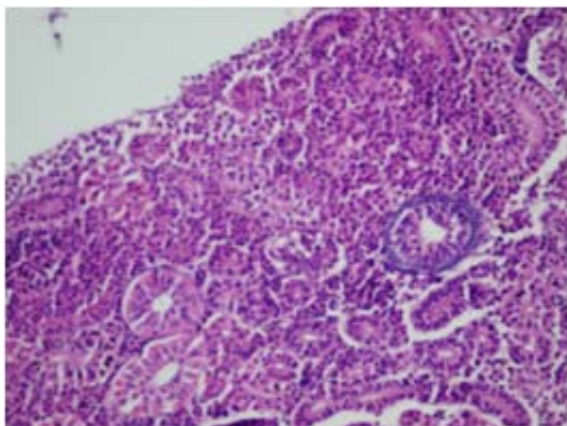


Fig. 4(a) : Kidney of control group (HE. x40)

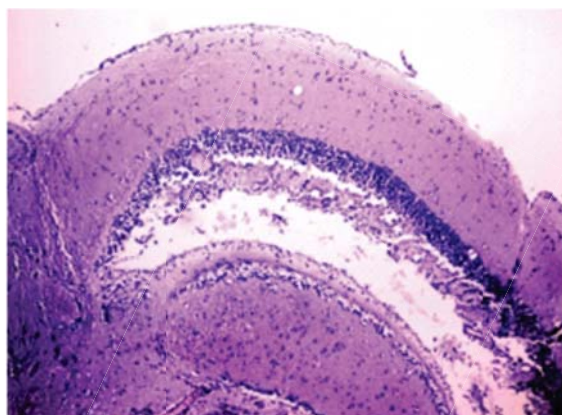


Fig. 5(a) : Brain of control group (HE. x40)

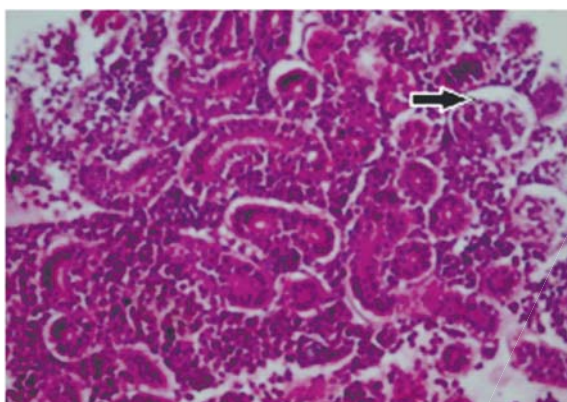


Fig. 4(b) : Enlargement of Cavum glomeruli and Bowman glomerulie (→)(HE. x40)

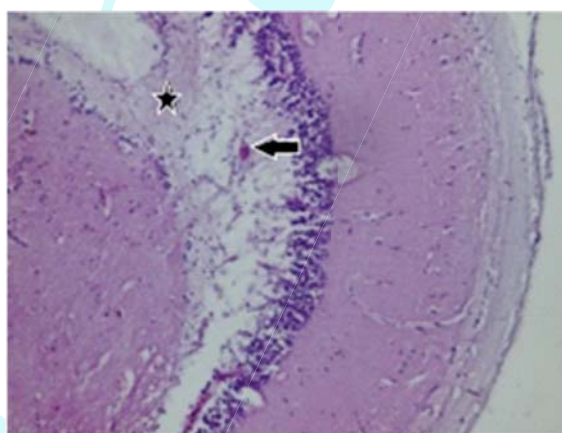


Fig. 5(b) : Hyperemia in brain (→), Edema (*) (HE. x20)

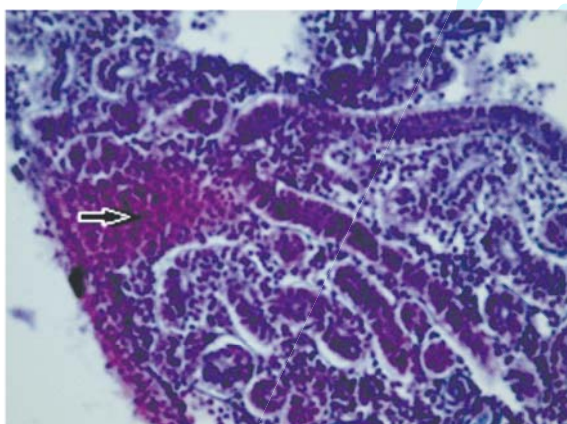


Fig. 4(c) : Hemorrhage in kidney (HE. x40)

Histological anomalies in the gill structure, which included lamellar lifting, edema, proliferation of the glandular cells and epithelium, covering the gill filament, fusion and degenerative alterations were observed in *Cyprinus carpio* exposed fungicide contamination

(Stoyanova, 2015). Gül *et al.* (2012) found hyperemia, telangiectasis, fusion in secondary lamella and hyperplasia, bronchitis and epithelial lifting in gills of carp (*Cyprinus carpio*) exposed to Propoxuron. Futher, hemorrhage, destruction in glomerulus, pre-nephritis, and edema in kidneys have also noted. Histopathological changes in gills, liver, gonads, muscles and skin of *Cyprinus carpio* exposed to sub lethal doses of insecticide phenyltrothion (Sepici Dinçel *et al.*, 2009).. Mikula *et al.* (2008) reported that the highest destruction was observed in gills and liver of the carp fingerlings exposed to various pesticides. Symptoms such as hygrophyte degeneration in hepatocytes, fattening, hemorrhage and cellular necrosis in liver and cellular dilatation and cellular proliferation in glomerules and necrosis in kidneys of rain bow trout exposed to cypermethrin has been reported. It was emphasized that kidney lesions should be evaluated together with the symptoms observed in other organs (Lawrence and Hemingway, 2003). In this investigation, permethrin caused important toxic effect of *P. parva* and the biggest damage was observed in gills, liver, and kidneys of the fish respectively. The present study

showed that hematological, histopathological and genotoxic biomarkers of toxicity in fish organs are a useful indicator of environmental pollution.

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