



Full Length Research Paper

Acute intoxication by deltamethrin in jundia: emphasis on clinical, biochemical and haematological effects

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Deltamethrin (DM) is a synthetic pyrethroid pesticide highly toxic to aquatic organisms. Because of its lipophilic feature it can be highly absorbed by the fish gills, which partially explains the high sensitivity of these animals to DM exposure in concentrations up to a thousand times lower than in mammals. This study compared clinical, biochemical and haematological observations between DM intoxicated and non-intoxicated fish. The experiment involved five exposure conditions (control and four concentrations of DM) and was executed in triplicates with 4 animals per set (n=60). The first behavior changes represented by rapid operculum movement and irregular or on the surface swimming were observed shortly after exposure to the concentrations of 0.5, 1.0 and 1.5 mg/L of DM. Fish exposed to the DM concentration (0.5 mg/L) for 96 h showed significantly higher leukocyte counts when compared to fish in the other groups. The response of the fish exposed to different concentrations of DM produced an inverted U-shaped curve, so the fish exposed to higher concentrations of DM may have had adaptive behavior alterations or loss of selectivity effects at concentrations high in relationship of leukocytes number. Significant decrease in the activity levels of some of the hepatic enzymes such as aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase result from DM exposure. The results demonstrated that DM environmental pollution can rapidly cause death to *Rhamdia quelen* as it is a toxic insecticide for this species.

Keywords: Fish, Toxicity, Hepatic enzymes, Leukocytes, Pyrethroids, *Rhamdia quelen*.

INTRODUCTION

The growing use of synthetic insecticides is intensifying global pollution risks. Insecticides are toxic and were designed to repel or kill unwanted organisms and when

used for their different purposes they may be brought to water bodies killing or influencing the lives of aquatic organisms (EL-SAYED et al., 2007). The effects of the use of insecticides are recognized worldwide and compounded by their improper use (TSUDA, 1995; Wilson and Tisdell, 2001).

Deltamethrin (DM) and other pyrethroids have proven to be toxic to aquatic organisms, mainly to fish. Due to its lipophilic characteristics it can be highly absorbed by the

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fish gills, which partially explains the high sensitivity of these animals to DM exposure in concentrations up to a thousand times lower than in mammals (Rodrigues, 2003).

The evaluation of blood components assists in diagnosing adverse conditions and in understanding the relationship between blood characteristics, the health status of fish and their association with the environment (Tavares-Dias and Moraes, 2004).

Blood analysis and biochemical parameters are used in aquatic medicine as an indicator of health for various stress conditions Pimpão et al. (2007). It is known that these analyses have meaningful value in toxicological assessments since the changes become apparent much earlier than the appearance of clinical symptoms produced by toxic agents (Rao, 2006).

According to Barcellos et al. (2004) the evaluation of blood cells and serum biochemistry can be useful for the diagnosis of diseases and to monitor the physiological state of the fish. The activity measurement of enzymes such as ALT (alanine aminotransferase) and AST (aspartate aminotransferase) has already been used in fish to demonstrate hepatic tissue damage Borges (2005).

This study aims at the hematological and biochemical evaluations of *Rhamdia quelen* exposed to sub lethal concentrations of DM in water for 96 hours, with emphasis on the assessment of hepatic tissue damage.

MATERIAL AND METHODS

The species chosen for the study is a riverine fish native in southern Brazil, named *Rhamdia quelen* and commonly known as Jundia. The fish were farmed in the Pisciculture and Research Laboratory (LAPEP) of the Pontifical Catholic University of Parana (PUCPR) where all the experimental tests were carried out in March 2009. This experiment was approved by Ethical Committee for Animal Use in Research of the PUCPR (process number 330).

The fish were kept in tanks with 1000 liters of volume for a period of 15 days. These tanks were kept with aeration and biological filtration, constant temperature (24-26 °C), pH 7 and 12 hour light/dark cycles. During this period the fish were fed with commercial feed once a day. After this period, they were measured and weighed, showing averages of 14 ± 3.2 cm and 40 ± 5 g respectively. The fish were later randomly distributed in 30 liter volume tanks involving five experimental treatments (control and four concentrations of DM, previously determined on the same laboratory). Each experimental treatment was executed in triplicate and each triplicate contained four animals in each tank ($n = 60$).

The animals were exposed to DM for 96 hours and the concentrations used were: 0; 0.1; 0.5; 1.0 and 1.5 mg/L.

At 24, 48, 72 and 96 hours any fish found dead was immediately removed; behavioral changes, clinical signs and *post-mortem* lesions were reported daily. A sub lethal concentration was identified previously in our laboratory at 96 hours was between 1.0 and 1.5 mg.L⁻¹ and the lethal concentration was 1.7 mg.L⁻¹.

Blood samples were collected in the volume of 0.8 mL through caudal vein puncture using syringes washed with EDTA 3%. Blood smears were stained according to the Rosenfeld (1947) method to assess the differential leukocyte count, morphological evaluation, visualization of thrombocyte aggregates and search for hemoparasites. Erythrocyte, leukocyte and thrombocyte counts were determined manually using a Neubauer counting chamber in an optical microscope after 1:200 blood dilutions in Natt/Herrick's (1952) dye. The hematocrit was determined using capillary tubes in a micro-hematocrit centrifuge (SISLAB/MH) operated at 11,000 rpm for five minutes. Total plasmatic protein was evaluated through a refractometer (KERNCO – OS1270). The hemoglobin content was determined spectrophotometrically (Fanem – Excelsa Baby II – mod. 206-R) through a cyanmethemoglobin reaction after centrifugation at 3,500 rpm for five minutes and expressed in g/dL.

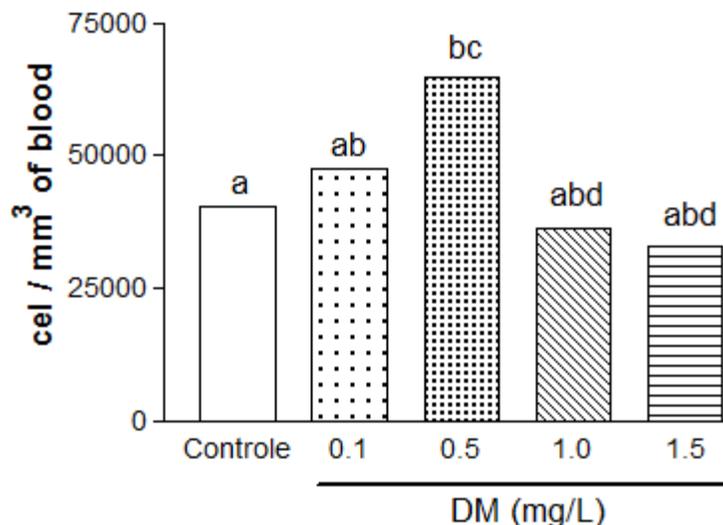
Aliquots of blood with the approximate volume of 0.5 mL were centrifuged at 3500 RPM for 5 minutes using a refrigerated centrifuge in order separate serum for the biochemical analyses.

The following biochemical tests were conducted by spectrophotometry (Drake – mod. Quick lab/Siel-mod. EPECTROMATIC 710): aspartate aminotransferase (AST-IU/L-Labtest liquiform), alanine aminotransferase (ALT-IU/L-Labtest liquiform), alkaline phosphatase (ALP-IU/L-Labtest liquiform), gamma-glutamyltransferase (GGT-IU/L-Labtest liquiform) and albumin (ALBUMIN-g/dL – colorimetric Labtest).

Statistical data analyzes were executed using ANOVA followed by the Bonferroni test for comparisons between groups using the statistical *Software GraphPad Prism* version 3 for Windows, San Diego-California, USA. Data is presented as averages \pm standard deviations.

RESULTS AND DISCUSSION

Daily evaluations of behavior changes in the species used were recorded. Animals in the control group and in the group exposed to the lowest concentration of DM (0.1 mg/L) did not present behavioral changes. The first behavior changes represented by rapid operculum movement and irregular or on the surface swimming were observed shortly after exposure to the concentrations of 0.5, 1.0 and 1.5 mg/L of DM. Fish were less active or inactive, remaining vertically in the water or laid on one side and in some cases still at the bottom of the tank just moments before death. *Post-mortem* signs observed in



Same letters mean $p > 0,05$; different letters mean $p < 0,05$;
DM - deltamethrin

Figure 1. Different DM concentrations in the assessment of total leukocyte counts

the animals exposed to DM were mainly darkening of the surface of the body, tail and wattles erosion and hemorrhagic spots on the body surface.

Many physiological changes that arise from environmental disturbances are usually used to detect stress in fish. Stress responses are identified by multiple behavior, hematological and biochemical changes. The main behavior changes observed in this study were represented by respiratory and neurological manifestations. Similar observations have been reported in "guppy" (*Poecilia reticulata*) VIRAN et al. (2003) and YILMAZ et al. (2004); in catfish (*Heteropneustes fossilis*) Saha and Kaviraj (2003); in common carp (*Cyprinus carpio*) Svobodova et al. (2003) and Çalta and Ural (2004); Nile tilapia (*Oreochromis niloticus*) Boateng et al. (2006) and rainbow trout (*Onchorhynchus mykiss*) Ural and Saglam (2005) and Velisek et al. (2007) when intensely exposed to various concentrations of synthetic DM and cypermethrin (pyrethroid similar to DM). These changes can be attributed to the neurotoxic effect of DM by blocking sodium channels and inhibiting the Gaba receptors in the nervous filaments which results in an excessive stimulation of the central nervous system that sometimes can lead to brain hypoxia (El-Sayed et al., 2007).

The toxic signs of darkening of the surface of the body, tail and wattles erosion, hemorrhagic spots on the body surface, irregular or on the surface swimming and rapid operculum movement described here have also been reported for "guppy" (*Poecilia reticulata*) Yilmaz et

al. (2004) and rainbow trout (*Onchorhynchus mykiss*) Ural and Saglam (2005), and can be assigned to the DM irritant effect (WHO, 1999).

After 96 hours of acute DM exposure the fish exposed to 0.5 mg/L presented a significant increase in the total leukocyte counts when compared to the control group ($p < 0.05$) and to the group exposed to 1.0 and 1.5 mg/L ($p < 0.01$). The values from the control group and from the group exposed to 0.1, 1.0 and 1.5 mg/L were similar to each other (Figure 1). In accordance with Figure 1 shows that the response of the fish exposed to different concentrations of DM produced an inverted U-shaped curve, so the fish exposed to higher concentrations of DM may have had adaptive behavior alterations or loss of selectivity effects at concentrations high.

The main haematological response of Jundia to the effect of acute DM intoxication in this study was a significant increase in the total leukocyte counts compared to the control group and is in agreement with the results reported by El-Sayed et al. (2007) in Nile tilapia (*Oreochromis niloticus*) and Pimpão et al. (2007) in catfish (*Ancistrus multispinis*). EL-Sayed et al. (2007) also reported lymphocytosis and neutropenia in the same study with Nile tilapia (*Oreochromis niloticus*). Sopinska and GUZ (1998) observed a decrease in the total leukocyte and neutrophil counts in common carp (*Cyprinus carpio*) when exposed to toxic doses of permethrin (pyrethroid similar to DM). The leukocytosis shows that this pesticide can generate inflammatory or stress responses. In the present study no significant diff-

Table 1. Averages and standard deviations of the leukocyte values

Parameters	Deltamethrin Concentrations				
	Control	0.1 mg/L	0.5 mg/L	1.0 mg/L	1.5 mg/L
Lymphocytes (%)	31.0 ± 5.34 ^a	43.92 ± 4.37 ^a	32.08 ± 6.08 ^a	24.82 ± 3.72 ^a	25 ± 5.98 ^a
Monocytes (%)	9.33 ± 1.44 ^a	7.08 ± 1.09 ^a	6.58 ± 0.85 ^a	8.81 ± 1.48 ^a	6.40 ± 1.28 ^a
Eosinophils (%)	1.14 ± 0.14 ^a	1.5 ± 0.28 ^a	2.0 ± 0.57 ^a	1.83 ± 0.47 ^a	1.50 ± 0.50 ^a
Neutrophils (%)	59 ± 5.49 ^a	48.58 ± 4.26 ^a	61.25 ± 5.84 ^a	65.36 ± 4.04 ^a	67.75 ± 6.90 ^a
Total leukocytes count (FreeBSD ³ /μl)	40.50 ± 7.45 ^a	47.63 ± 6.09 ^{ab}	64.92 ± 4.70 ^b	36.36 ± 4.46 ^a	32.80 ± 2.15 ^a
Total thrombocytes count (FreeBSD ³ /μl)	52.46 ± 5.96 ^a	36.42 ± 5.26 ^a	43.92 ± 7.72 ^a	58.68 ± 4.79 ^a	43.60 ± 10.30 ^a

Same letters mean $p > 0.05$; different letters mean $p < 0.05$.

Table 2. Averages and standard deviations of the erythrocyte values

Parameters	Concentration Deltamethrin				
	Control	0.1 mg/L	0.5 mg/L	1.0 mg/L	1.5 mg/L
Erythrocytes (x 10 ⁶ /μl)	1.48 ± 0.094 ^a	1.33 ± 0.134 ^a	1.32 ± 0.085 ^a	1.52 ± 0.098 ^a	1.30 ± 0.176 ^a
Haematocrit (%)	29.50 ± 1.95 ^a	26.73 ± 1.51 ^a	26.83 ± 1.07 ^a	31.18 ± 2.11 ^a	25.60 ± 1.40 ^a
Hemoglobin (g/dL)	6.01 ± 0.40 ^a	5.16 ± 0.32 ^a	5.50 ± 0.2 ^a	5.68 ± 0.27 ^a	5.39 ± 0.26 ^a
Plasma Protein (g/dL)	4.53 ± 0.36 ^a	4.16 ± 0.27 ^a	4.10 ± 0.22 ^a	3.85 ± 0.29 ^a	3.76 ± 0.29 ^a

Same letters mean $p > 0.05$

erence was observed in the differential leukocyte counts which is in agreement of the results reported by Velisek et al. (2007) in rainbow trout (*Onchorhynchus mykiss*) intoxicated with DM.

There was no significant difference between groups as for the number of erythrocytes, hemoglobin, hematocrit, plasmatic protein, neutrophils, eosinophils, lymphocytes, monocytes and thrombocytes (Tables 1 and 2).

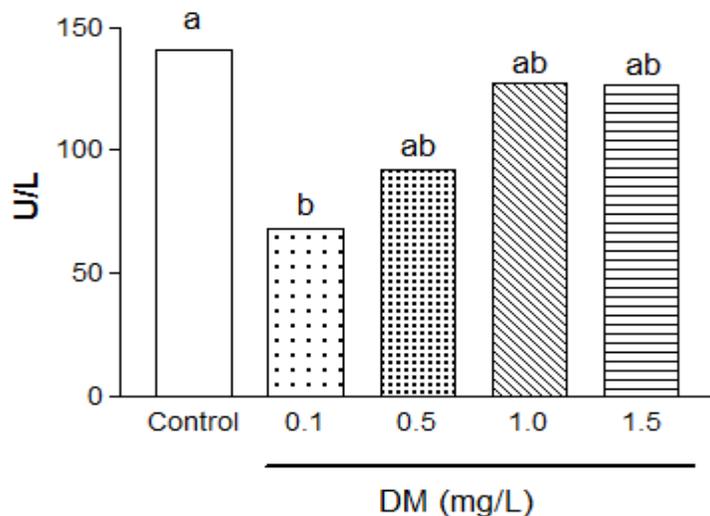
Velisek et al. (2006) described a significant decrease in the total thrombocyte counts in rainbow trout exposed to cypermethrin (pyrethroid similar to DM). In this study, no significant difference was found in the thrombocyte counts between the groups. This difference in results could possibly result from the different methodology employed for blood collection and the degree of stress in the fish at the time of collection since glucocorticoids are important effectors in the reduction of the quantity and quality of thrombocytes (Campbell, 1998).

Changes in the values for erythrocytes, hemoglobin, hematocrit rate and plasmatic protein were not significant in this experiment, unlike the results in a study by Velisek et al. (2007) where a significant increase of these parameters is reported. Svobodova et al. (2003) describe a significant decrease in the total erythrocyte counts in the common carp (*Cyprinus carpio*) and in rainbow trout (*Onchorhynchus mykiss*) when intoxicated with DM. Velisek et al. (2006) reported a significant increase in the total erythrocyte counts in rainbow trout. Pimpão et al.

(2007) observed an increase in the total erythrocyte counts in catfish (*Ancistrus multispinis*) intoxicated with DM while El-Sayed et al. (2007) observed a significant increase in erythrocytes, hemoglobin and hematocrit rate in Nile tilapia (*Oreochromys niloticus*) exposed to this same toxic substance. These changes can be attributed to the mechanisms involved in the reactivation of erythropoiesis induced by the spleen and liver in response to the cerebral hypoxia caused by DM exposure (Pimpão et al., 2007).

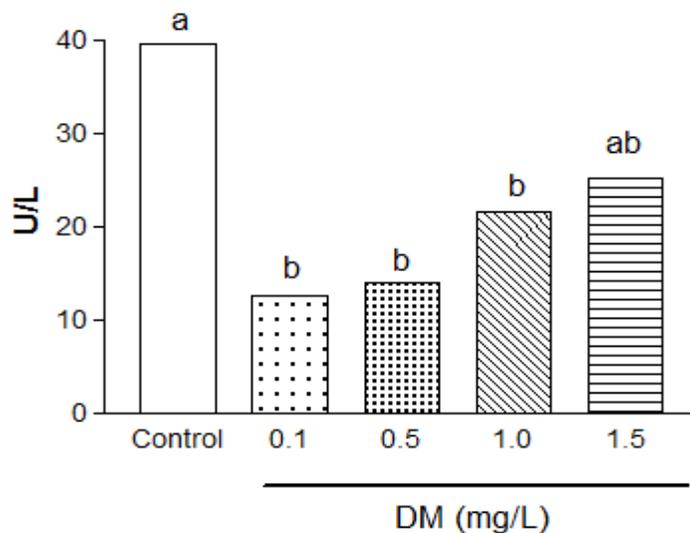
The results obtained showed that the DM has not changed the values of albumin significantly though showed a tendency to increase in the fish exposed to the higher concentrations (1.0 and 1.5 mg/L). No statistically significant difference was observed in the albumin levels between the tested groups and the control group in this study. Similar results were observed by Velisek et al. (2006) with rainbow trout (*Onchorhynchus mykiss*) exposed to cypermethrin (similar to DM pyrethroid). However an increase in the levels of this biochemical parameter was observed in a similar study using DM (Velisek et al., 2007). Nevertheless El-Sayed et al. (2007) studying Nile tilapia (*Oreochromys niloticus*) exposed to DM and Nayak et al. (2004) studying "rohu" (*Labeo rohita*) exposed to permethrin (pyrethroid similar to DM) reported decreased levels for this serum protein.

The values for aspartate aminotransferase (AST) from the group exposed to 0.1 mg/L DM decreased significan-



Same letters mean $p > 0,05$; different letters mean $p < 0,05$; DM - deltamethrin

Figure 2. Different DM concentrations in the assessment of the aspartate aminotransferase (AST) values

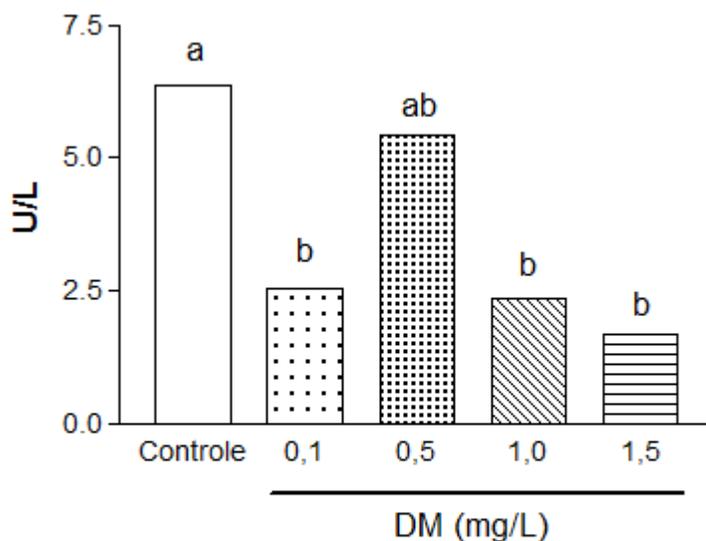


Same letters mean $p > 0,05$; different letters mean $p < 0,05$; DM - deltamethrin

Figure 3. Different DM concentrations in the assessment of the Alanine Aminotransferase (ALT) values

tly compared to the control group ($p < 0.05$), with a tendency to increase in the groups exposed to 0.5, 1.0 and 1.5 mg/L (Figure 2). A significant reduction in the values of alanine aminotransferase (ALT) was observed in the groups exposed to 0.1 and 0.5 mg/L ($p < 0.01$) and 1.0 mg/L ($p < 0.05$) compared to the control group. This enzyme's levels are likely to increase in the groups

exposed to 1.0 and 1.5 mg/L (Figure 3). The ALT and AST enzymes are the most important aminotransferases related to the metabolism of amino acids in the liver of teleost fish (Coz Rakovac and SMUC, 2008). The ALP and GGT enzymes are important in the detection of cell membrane destruction in the hepatocytes (Kramer and Hoffmann, 1997). The existence of ALP isoenzymes such



Same letters mean $p > 0,05$; different letters mean $p < 0,05$;
DM - deltamethrin

Figure 4. Different DM concentrations in the assessment of the Alkaline Phosphatase (FA) values

Table 3. Averages and standard deviations for the biochemical parameters

Parameters	Deltamethrin Concentration				
	Control	0.1 mg/L	0.5 mg/L	1.0 mg/L	1.5 mg/L
AST (IU/L)	140.90 ± 24.30 ^a	68.18 ± 1.69 ^b	91.67 ± 12.50 ^{ab}	127.30 ± 14.45 ^{ab}	126.30 ± 12.70 ^{ab}
ALT (IU/L)	39.61 ± 7.95 ^a	12.44 ± 2.84 ^b	14.02 ± 2.02 ^b	21.46 ± 2.64 ^b	25.21 ± 3.23 ^{ab}
FA (IU/L)	6.36 ± 1.39 ^a	2.52 ± 0.60 ^b	5.43 ± 0.80 ^{ab}	2.36 ± 0.50 ^b	1.68 ± 0.23 ^b
GGT (IU/L)	2.83 ± 0.19 ^a	3.02 ± 0.83 ^a	2.08 ± 0.34 ^a	1.73 ± 0.48 ^a	2.49 ± 1.03 ^a
ALBUMIN (g/dL)	0.76 ± 0.02 ^a	0.79 ± 0.02 ^a	0.78 ± 0.03 ^a	0.86 ± 0.02 ^a	0.85 ± 0.09 ^a

Same letters mean $p > 0.05$; different letters mean $p < 0.05$.

as observed in mammals is not known in fish (Hrubec et al., 2001).

A reduction in the alkaline phosphatase values (ALP) was observed in the groups exposed to 0.1 mg/L ($p < 0.05$), 1.0 and 1.5 mg/L ($p < 0.01$) when compared to the levels observed in the control group. However a reduction in the alkaline phosphatase values (ALP) was also observed in the group exposed to 1.5 mg/L ($p < 0.05$) when compared to the group exposed to 0.5 mg/L. The statistical analysis showed a tendency to increased levels in the group exposed to 0.5 mg/L DM (Figure 4). According to the data obtained no difference was observed between the groups for the gamma-glutamyltransferase (GGT) parameter (Table 3).

The AST is not a specific liver enzyme, but when organs are damaged and no tissue necrosis, a release for serum AST. Already ALT has the highest

concentration in the liver, and its evaluation more useful in the investigation of liver disease. This enzyme is released by the liver after the destruction of liver tissue and blood found quickly. In this study the decrease of serum levels of ALT, AST and ALP were observed. Significant increase of enzymatic activity of AST, ALT and ALP as a result of acute DM intoxication have been reported by Rao (2006) in studies with Mozambican tilapia (*Oreochromys mossambicus*) and by El-Sayed et al. (2007) with Nile tilapia (*Oreochromys mykiss*). Increase of ALT and AST levels were also observed in "rohu" (*Labeo rohita*) intoxicated with permethrin (pyrethroid similar to DM) (Nayak et al., 2004). Velisek et al. (2006) reported increased AST, decreased ALP and unchanged ALT values in rainbow trout (*Onchorhynchus mykiss*) exposed to cypermethrin (pyrethroid similar to DM). Conversely, Velisek et al. (2007) observed a signifi-

cant increase in the AST levels and ALT decreased levels in rainbow trout (*Onchorhynchus mykiss*) exposed to DM. In this study no significant changes were observed in the GGT serum levels. Other comparative studies do not refer to the evaluation of this biochemical parameter.

Acute exposure to deltamethrin in rainbow trout was associated with a significantly ($p < 0.05$) lower concentration of GLU, ALT, and significantly ($P < 0.05$) greater of AST compared to controls. The common carp exposed to deltamethrin exhibited significantly higher ($p < 0.05$) value of AST, and ALT compared to controls. Acute exposure to cypermethrin resulted in a significantly ($p < 0.01$) lower concentration of ALP and significantly ($p < 0.01$) higher concentration of AST in rainbow trout compared to controls fish. In common carp cypermethrin resulted in a significant ($p < 0.01$) lower in ALP (Velisek et al., 2011).

CONCLUSION

According to the data obtained from the biochemical tests we can observe a compromised liver function in Jundiá translated by tissue responses in all of the fish exposed to DM independent of the concentration used.

The evaluation of the haematological parameters indicates leukocytosis as a cellular response in the fish exposed to the lower DM concentration (0.5 mg/L) used.

The study of blood characteristics can provide important benefits for the diagnosis and prognosis of morbid conditions in fish populations besides contributing to understand the relationships between the physiology of these populations and the ecological parameters.

REFERENCES

- Barcellos L, Kreutz L, Souza C, Rodrigues L, Fioreze I, Quevedo R, Cericato L, Soso A, Fagundes M, Conrad J (2004). Hematological changes in jundiá (*Rhamdia quelen* Quoy and Gaimard *Pimelodidae*) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. *Aquaculture*, v. 237, p. 229-236
- Boateng JON, Nunoo FKE, Dankwa HR, Ocran MH (2006). Acute toxic effects of deltamethrin on tilapia, *Oreochromis niloticus* (Linnaeus, 1758). *West Afr. J. Appl. Ecol.* v. 9, p. 1-18
- Borges A (2005). Valores hematológicos e bioquímicos séricos, efeitos de doses sub-letais da cipermetrina e características físico-químicas do sêmen do Jundiá *Rhamdia quelen*, Universidade Federal do Rio Grande do Sul. Instituto de Ciências Básicas da Saúde. Programa de Pós-Graduação em Ciências Biológicas: Fisiologia.
- Çalta M, Ural M (2004). Acute toxicity of the synthetic pyrethroid deltamethrin to young mirror carp, *Cyprinus carpio*. *Fresen. Environmental Bulletin*, v. 13, p. 1179-1183,
- Campbell T (1998). Tropical fish medicine. Fish cytology and hematology. The Veterinary clinics of North America Small animal practice, v. 18, p. 349
- Coz Rakovac R, Smuc T (2008). Novel methods for assessing fish blood biochemical data. *J. Appl. Ichthyol.* v.24, p.77-80
- El-Sayed YS, Saad TT, El-Bahr SM (2007). Acute intoxication of deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical, biochemical and haematological effects. *Environmental Toxicology and Pharmacology*, v. 24, p.212-217
- Hrubec TC, Smith SA, Robertson JL (2001). Age-related Changes in Hematology and Plasma Chemistry Values of Hybrid Striped Bass (*Morone chrysops* × *Morone saxatilis*). *Veterinary Clinical Pathology*, v.30, p. 8-15
- In Tech publishers, 614p
- Kramer J, Hoffmann W (1997). Chapter 12: Clinical Enzymology. *Clinical Biochemistry of Domestic Animals*. 5th ed. Kaneko J.J.; Harvey, J.W.; Bruss, M.L., ed. Academic Press, London, UK, 316 p
- Natt MP, Herrick CA (1952). A new blood diluent for counting the erythrocytes and leucocytes of the chicken. *Poult. Science*, v. 31, p. 735-739,
- Nayak A, Das B, Kohli M, Mukherjee S (2004). The immunosuppressive effect of [alpha]-permethrin on Indian major carp, rohu (*Labeo rohita* Ham.). *Fish and Shellfish Immunology*, v. 16, p. 41-50
- Pimpão CT, Zamprônio AR, Silva De Assis HC (2007). Effects of deltamethrin on hematological parameters and enzymatic activity in *Ancistrus multispinis* (Pisces, Teleostei). *Pesticide Biochemistry and Physiology*, v. 88, p. 122-127
- Rao J (2006). Biochemical alterations in euryhaline fish, *Oreochromis mossambicus* exposed to sub-lethal concentrations of an organophosphorus insecticide, monocrotophos. *Chemosphere*, v. 65, p.1814-1820,
- Rodrigues LC (2003). Estudo das Glutathion S-Transferases hepáticas solúveis do peixe *Piaractus mesopotamicus* Holmberg, 1887 (Pacu). Tese Doutorado, Pós-Graduação em Biologia do Instituto de Biologia Roberto Alcântara Gomes / UERJ, Rio de Janeiro, RJ
- Rosenfeld G (1947). Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido. *Memórias do Instituto Butantan*, v. 20, p.329-334
- Saha S, Kaviraj A (2003). Acute Toxicity of Synthetic Pyrethroid Cypermethrin to Freshwater Catfish *Heteropneustes fossilis* (Bloch). *Int. J. Toxicol.* v. 22, p.325-328
- Sopinska A, Guz L (1998). Wpływ Permetryny Na Aktywnosc Fagocytow Karpia= Influence of Permethrin on Phagocytic Activity of Carp. *Medycyna Weterynaryjna*, v. 54, p.126-128
- Svobodova Z, Luskova V, Drastichova J, Svoboda M, Zlabek V (2003). Effect of Deltamethrin on Haematological Indices of Common Carp (*Cyprinus carpio* L.). *Acta Veterinaria*, v. 72, p.79-85.
- Tavares-Dias M, Moraes F (2004). Hematologia de peixes teleósteos. Editora Eletrônica e Arte Final, 144 p.
- Tsuda T, Aoki S, Inoue T, Kojima M (1995). Accumulation and excretion of diazinon, fenthion and fenitrothion by killifish: Comparison of individual and mixed pesticides. *Water Research*, v. 29, p. 455-458
- Ural M, Saglam N (2005). A study on the acute toxicity of pyrethroid deltamethrin on the fry rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). *Pesticide Biochemistry and Physiology*, v. 83, p.124-131,
- Velisek J, Jurčíková J, Dobsíková R, Svobodová Z, Piacková V, Máchová J, Novotný L (2007). Effects of deltamethrin on rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Pharmacology*, v.23, p.297-301
- Velisek J, Stara A, Svobodova Z (2011). The Effects of Pyrethroid and Triazine Pesticides on Fish Physiology in STOYTCHÉVA, M. *Pesticides in the Modern World*.
- Velisek J, Wlasow T, Gomulka P, Svobodova Z, Dobsikova R, Novotny L, Dudzik M (2006). Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). *Veterinary Medicine*, v.51, p. 469-476,
- Viran R, Ünlü Erkoç F, Polat H, Koçak O (2003). Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). *Ecotoxicology and Environmental Safety*, v. 55, p. 82-85

WHO, Report of the Third WhopesWorking Group Meeting. Review of: Deltamethrin 1% SC and 25% WT Etofeprox 10% EC and 10% EW. World Health Organization, v. 3, p. 23-24, 1999.

Wilson C, Tisdell C (2001). Why farmers continue to use pesticides despite environmental, health and sustainability costs. Ecological Economics, v 39, p. 449-462

Yılmaz M, Gül A, Erbasli K (2004). Acute toxicity of alpha-cypermethrin to guppy (*Poecilia reticulata*, Pallas, 1859). Chemosphere, v. 56, p. 381-385