ARS VETERINARIA, Jaboticabal, SP, v.37, n.2, 067-073, 2021. ISSN 2175-0106
DOI: http://dx.doi.org/10.15361/2175-0106.2021v37n2p67-73

CLINICAL SAFETY OF ZAFIRLUKAST TREATMENT DURING ACUTE INFLAMMATORY REACTION IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

SEGURANÇA CLÍNICA DO TRATAMENTO COM ZAFIRLUCASTE DURANTE A REAÇÃO INFLAMATÓRIA AGUDA EM TILÁPIA DO NILO (OREOCHROMIS NILOTICUS)

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SUMMARY

Little is known about the toxicity of immune modulators in fish. Zafirlukast is an anti-inflammatory that antagonizes cysteine leukotriene receptors (CysLTR1). Aiming to study immunomodulatory treatments on fish health, this study evaluated the clinical safety of oral zafirlukast treatment, through biochemical and hematological analyzes during acute inflammatory reaction in Nile tilapia (*Oreochromis niloticus*), induced by *Aeromonas hydrophila* bacterins. 72 young tilapias were randomly divided in 9 aquariums (100 L each, n=8) to compose the following treatments: T0 (control), T1 (Treatment with 250 µg zafirlukast) and T2 (Treatment with 500 µg zafirlukast). Eight animals were evaluated per treatment in three periods: six, 24 and 48 hours post-inoculation (HPI), blood collection was performed for hematological and serum biochemical evaluation. The study of hepatic and renal functionality revealed that treatment with both doses of zafirlukast did not result in changes in the circulating values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, creatinine, triglycerides, cholesterol, and total protein, suggesting that the drug has not presented hepatotoxicity, as well as compromised liver and kidney functions. Tilapia submitted to treatment with 500 µg showed adverse hematological effects characterized by polycythemia associated with microcytosis. Therefore, oral treatment with zafirlukast has demonstrated clinical safety at a therapeutic dose of 250 µg in tilapia during acute aerocystitis, although hematological changes were observed in tilapia treated with overdose of this leukotriene blocker.

KEY-WORDS: Cichlids. Acute inflammation. Toxicity. Cysteine leukotrienes. Hematology.

RESUMO

Pouco se sabe sobre a toxicidade de imunomoduladores em peixes. Zafirlukast é um anti-inflamatório que antagoniza os receptores de leucotrienos cisteínicos (CysLTR1). Com o objetivo de estudar o efeito de tratamentos imunomoduladores sobre a saúde dos peixes, este estudo avaliou a segurança clínica do tratamento com zafirlucaste oral, por meio de análises bioquímicas e hematológicas durante reação inflamatória aguda em tilápia do Nilo (*Oreochromis niloticus*), induzida por bacterinas de *Aeromonas hydrophila*. Para tal, 72 tilápias jovens foram divididas aleatoriamente em 9 aquários (100 L cada, n=8) para compor os seguintes tratamentos: T0 (controle), T1 (Tratamento com 250 µg de zafirlucaste) e T2 (Tratamento com 500 µg de zafirlucaste). Oito animais foram avaliados por tratamento em três períodos: seis, 24 e 48 horas pós-inoculação (HPI), foi realizada coleta de sangue para avaliação hematológica e bioquímica sérica. O estudo da funcionalidade hepática e renal revelou que o tratamento com ambas as doses de zafirlucaste não resultou em alterações nos valores circulantes de aspartato aminotransferase (AST), alanina aminotransferase (ALT), fosfatase alcalina, creatinina, triglicerídeos, colesterol e proteína total, sugerindo que a droga não comprometeu as funções hepáticas e renais. As tilápias tratadas com 500 µg apresentaram efeitos hematológicos adversos caracterizados por policitemia associada a microcitose. Portanto, o tratamento oral com zafirlucaste demonstrou segurança clínica na dose de 250 µg em tilápias durante aerocistite aguda, embora alterações hematológicas tenham sido observadas em tilápias tratadas com sobredosagem deste bloqueador de leucotrieno.

PALAVRAS-CHAVE: Ciclídeos. Inflamação aguda. Toxicidade. Leucotrienos cisteínicos. Hematologia.

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Submetido: 29/04/2021 Aceito: 01/06/2021

INTRODUCTION

Brazilian fish farming grew 4.9% in 2019, with tilapia as the main species responsible for 57% of all national production, in addition to maintaining Brazil as the 4th largest tilapia producer in the world (PeixeBR, 2020). Nile tilapia (*Oreochromis niloticus*) is one of the most widely reared species in aquaculture with great economic importance, and its production has increased intensively to meet the growing global demand for fishery products (FAO, 2020). In aquaculture systems, high stocking densities have been used to increase productivity. However, intensive fish production systems can raise stress levels, resulting in changes in physiological homeostasis, reduced growth rate and reproductive performance, as well as suppression in the immune system function making these animals more susceptible to disease (Ran et al., 2016).

According to Eto et al. (2020), teleost fish have demonstrated several advantages in relation to other animal species, being an alternative to the use of experimental models with rodents. They can provide additional information when used as a model for disease studies and prospecting for new drugs and vaccines (Charlie-Silva et al., 2020a; Farias et al., 2020). For decades, classic experimental models with mammals have been used to study the evolution of the inflammatory response in the acute or chronic phase. The model of aerocystitis, which mimics the pleurisy technique in rodents, proved to be extremely effective for the study of acute inflammation in teleost fish (Reque et al., 2010; Claudiano et al., 2013; Castro et al., 2014; Prado et al., 2018).

The inflammatory process and its control mechanisms are well known in mammals; recently, important advances have been made in this area of knowledge in bony fish (Rodrigues-Soraes et al., 2018; Charlie-Silva et al., 2020b). Teleost fish are free-living organisms that mainly depend on their innate immune system to survive (Magnadóttir, 2006). According to Dotta et al. (2008), vascular changes are the main characteristics of the acute phase of inflammation. Zafirlukast has anti-inflammatory activity by antagonizing the cysteine leukotriene receptors, CysLTR1, acting on smooth muscle contraction and preventing the increase in vascular permeability, thus reducing the formation of edema and diapedesis, in addition to having secondary anti-inflammatory activities, which seem to be particularly effective in targeting neutrophils and monocytes / macrophages (Scott & Peters-Golden, 2013). These secondary anti-inflammatory mechanisms of CysLTR1 antagonists have been studied in human medicine, but the effects of blocking these receptors in tilapia are not known.

Innocuousness is a relevant factor when studying the administration of pharmacological compounds. According to Klaassen (2013), every substance is potentially toxic, and the correct dose is what differentiates the medicine from the poison. On the other hand, the analysis of biochemical and hematological parameters can point out important information for diagnosis and prognosis of the morbid conditions of an individual or their population (Belo et al., 2013; 2014). In this context, little is known about the clinical safety of zafirlukast in bony fish. Therefore, this investigation has experimentally studied and identified the harmlessness of treatment with this leukotriene blocker in Nile tilapia (*Oreochromis niloticus*), through biochemical and hematological analyzes during aerocystitis by *Aeromonas hydrophila* bacterins.

MATERIAL AND METHODS

Fish and rearing criteria

72 Nile tilapias (weighing ± 100g) from the same spawning (genetically improved lineage from Aquabel, Porto Ferreira/SP) were stored in 9 aquariums (n = 8), with a capacity of 100 L of water, supplied with running water devoid of chlorine, from an artesian well with a flow rate of 1 L/min. After being transported to the aquariums, the fish were acclimated for 15 days, a time necessary for the plasma cortisol concentration and osmolarity to return for baseline levels. In the first three days of acclimatization, the animals were subjected to baths in NaCl solution at a concentration of 6.0g/L according to Carneiro and Urbinati (2001). The feeding of the animals was done twice a day at 08:00 and 17:00, corresponding to 2% of the biomass of the aquariums. The water quality was determined twice a day (at time of feeding), temperature and concentration of dissolved oxygen measured by the YSI device, model 55, and the pH and electrical conductivity by the YSI device, model 63. The research project was accepted by the Ethics Committee on the Use of Animals (CEUA) from Brazil University (UB) process number 0015/2016.

Experimental design

Tilapias were randomly divided in 9 aquariums (100 L each, n=8) to compose the following treatments: T0 (control), T1 (Treatment with 250 µg zafirlukast) and T2 (Treatment with 500 µg zafirlukast). Eight animals were evaluated per treatment in three periods: six, 24 and 48 hours post-inoculation (HPI) with *Aeromonas hydrophila* in the swim bladder for blood collection. Fish from treatments with 250 (T1) and 500 µg (T2) were fed with these diets for one week before inoculation of *A. hydrophila* bacterin. The feeding of the animals was carried out twice a day (8:00 am and 5:00 pm), with administration of 2% of the aquarium biomass.

Experimental diets

Tilapia were fed a commercial basal diet (Fri Acqua®), containing 32% crude protein (Control Group, T0). For treatments T1 and T2 (as outlined in item 2.2), the cysteine leukotriene receptor blocker (zafirlukast, Accolate®) was added at doses of 250 and 500µg/kg body weight (These doses were extrapolated from a pharmacokinetic study in mammals, Dekhuijzen & Koopmans, 2002). For the preparation of the diets, tilapias were weighed individually, and an average of each aquarium was made to determine the amount of feed to be administered per aquarium. The quantities of zafirlukast

(250 and 500µg / kg) were weighed and mixed with 2% vegetable oil for incorporation into the feed, stored in dark plastic bags, kept at -16ºC, until the moment of use. For standardization of diets, 2% vegetable oil was added to the diet of the control group (T0).

Anesthesia of fish

Tilapias were anesthetized by immersion in an aqueous solution of benzocaine in the proportion of 1: 10,000 for bacterin inoculation in the swim bladder and 1: 500 at the time of euthanasia. Benzocaine was diluted in 98 ° alcohol (0.1 g / mL), completing the volume to 1L (Wedemeyer, 1980). After experimental handling, the animals were replaced in the aquariums with continuous water flow and aeration.

Production of Aeromonas hydrophila bacterin

Isolates of *A. hydrophila* were supplied by LAPOA (Laboratory of Pathology of Aquatic Organisms), CAUNESP. The bacterial mass was obtained by centrifugation (4000 rpm, 4ºC, for 20 minutes), after three successive washes with sterile PBS solution (pH 7.4) for complete removal of the culture medium and then it was resuspended in 100 mL of PBS. The bacterin concentration was adjusted to 1.0 x 10^9 ml^{-1} cells. For inactivation, 0.5% formaldehyde (v/v) was added to the bacterial suspension, which remained under constant agitation at room temperature. Then being kept at 4ºC, for 24 hours. At the time of inoculation, 0.5 mL of the inoculum was administered in the swim bladder. Eight animals were evaluated per treatment in three periods, that is: 6, 24 and 48 HPI. Proper antisepsis with alcohol was performed before the procedure and subsequently 0.5 mL of the inoculum was administered to the tilapia's swim bladder.

Hematological analysis

Blood samples were collected from the fish by puncture of the caudal vessel. The determination of the global red cell count was performed in a Neubauer chamber, using Natt and Herrick's solution (1952) with diluent in the proportion of 1: 100. The determination of the percentage of hematocrit in a microcentrifuge and the amount of circulating hemoglobin with Drabkin´s reagent for reading at a wavelength of 540nm. The values of mean corpuscular volume (MCV) were obtained by calculating MCV = (Ht / He) * 100, hematocrit Ht, red blood cells He and mean corpuscular hemoglobin concentration (MCHC) by calculating MCHC = (Hg / Ht) * 100, hemoglobin (Hg), hematocrit Ht following methodology used by Farias et al. (2016).

Serum biochemical evaluation

Serum samples were used to determine the biochemical values of alkaline phosphatase, cholesterol, triglycerides, albumin, total protein, creatinine, Aspartate Aminotransferase AST and Alanine Aminotransferase ALT through enzymatic and colorimetric determination in a semi-automatic biochemical analyzer (Model LabQuest - Bioplus®).

Statistical analysis

The results were analyzed statistically by the factorial scheme 3 X 3 (3 treatments with anti-inflammatory and 3 different times), "Split Plot Design", using the GLM (General Linear Model) procedure of the SAS program, version 9.3 (Statistical Analysis Software, 2012). The analysis of variance of the means was determined by the Tukey test ($p < 0.05$), according to Snedecor & Cochran (1984).

RESULTS

Hematological analysis

Tilapia treated with 500 µg zafirlukast showed an increase in the number of circulating erythrocytes when compared to animals treated with 250 µg in 6 and 24 HPI, being associated with a significant decrease ($p < 0.05$) MCV in 6 and 24 HPI and in the percentage values of hematocrit at 48 HPI (Table 1). There were no significant changes ($p \geq 0.05$) in the mean values hemoglobin and MCHC between the different treatments (Table 1).

Table 1 - Mean values[1] (± SE) and ANOVA[2] of erythrocyte parameters during aerocystitis induced by *A. hydrophila* bacterins in tilapia treated with zafirlukast.

Period	Treatments [3]	Total Erythrocytes (10^6/mm^3)	Hematocrit (%)	Hemoglobin (g/dL)	MCV [5] (fL)	MCHC [5] (g/dL)
6 hours	Control	2.13 ± 0.16 ^{ABa}	24.62 ± 0.51 ^{Ab}	4.80 ± 0.22 ^{Aa}	129.40 ± 11.40 ^{Aa}	19.64 ± 0.30 ^{Aa}
	250 Mg	1.76 ± 0.05 ^{Ba}	26.87 ± 0.42 ^{Aa}	5.10 ± 0.08 ^{Aa}	145.89 ± 8.08 ^{Aa}	18.90 ± 0.21 ^{Aa}
	500 Mg	2.71 ± 0.21 ^{Aa}	26.77 ± 0;69 ^{Aa}	5.43 ± 0.37 ^{Aa}	106.65 ± 9.08 ^{Aa}	20.00 ± 1.06 ^{Aa}
24 hours	Control	2.13 ± 0.17 ^{ABa}	30.62 ± 1.17 ^{Aa}	4.57 ± 0.14 ^{Aa}	164.16 ± 19.37 ^{Aa}	15.11 ± 0.63 ^{Aa}
	250 Mg	1.64 ± 0.05 ^{Ba}	27.10 ± 1.06 ^{Aa}	4.82 ± 0.09 ^{Aa}	156.18 ± 4.32 ^{Aa}	17.67 ± 0.86 ^{Aa}
	500 Mg	2.88 ± 0.15 ^{Aa}	26.66 ± 0.98 ^{Aa}	4.70 ± 0.24 ^{Aa}	95.84 ± 5.25 ^{Ba}	17.72 ± 0.96 ^{Aa}
48 hours	Control	1.83 ± 0.09 ^{Aa}	26.55 ± 0.90 ^{ABab}	5.05 ± 0.19 ^{Aa}	147.16 ± 4.02 ^{Aa}	19.13 ± 0.79 ^{Aa}
	250 Mg	2.03 ± 0.09 ^{Aa}	30.77 ± 0.95 ^{Aa}	5.75 ± 0.18 ^{Aa}	155.75 ± 7.83 ^{Aa}	18.97 ± 0.90 ^{Aa}
	500 Mg	1.91 ± 0.02 ^{Ab}	25.22 ± 1.17 ^{Ba}	5.24 ± 0.24 ^{Aa}	139.77 ± 3.53 ^{Aa}	19.85 ± 0.68 ^{Aa}
Treatment [4]		12.70 **	2.12 ^{NS}	1.77 ^{NS}	8.56 **	1.07 ^{NS}
Time		2.78 ^{NS}	2.15 ^{NS}	4.17 *	2.01 ^{NS}	6.63 **
Treatment X Time		4.52 **	3.92 **	0.66 ^{NS}	1.78 ^{NS}	0.78 ^{NS}
C.V. [4]		23.75	13.26	16.31	26.30	15.90

[1] Means (n = 8) followed by the same letter do not differ by the Tukey test (P <0.05).
[2] Analysis of statistical variance represented by capital letters compare in the column the different treatments within each experimental day, lowercase letters compare in the column the evolution of each treatment in different experimental days.
[3] Control (inoculated and untreated); Treated with 250 and 500 µg of zafirlukast/kg of body weight.
[4] CV- Coefficient of Variation (%); NS - Not significant; * significant for P <0.05; ** Significant for P <0.01
[5] MCV – Mean corpuscular volume; MCHC - Mean corpuscular hemoglobin concentration;

Serum biochemical analysis

The analysis of liver cytoxicity of tilapia treated with zafirlukast and inoculated with *Aeromonas hydrophila* did not reveal significant changes in the serum enzymatic activity of AST and ALT in fish subjected to different treatments (Table 2). Fish treated with 250 µg zafirlukast showed a significant increase in the serum activity of alkaline phosphatase 48 HPI (Table 2).

Table 2 - Mean values[1] (± SE) and ANOVA[2] observed in the analysis of the enzymatic serum activity of alkaline phosphatase, AST and ALT in tilapia treated with zafirlukast and inoculated with *Aeromonas hydrophila* bacterins.

Period	Treatments [3]	Alkaline Phosphatase (U/L)	AST [5] (U/L)	ALT [5] (U/L)
6 hours	Control	21.09 ± 1.29 Aa	54.50 ± 8.95 Aa	7.85 ± 0.88 Aa
	250 µg	25.91 ± 2.51 Ab	47.13 ± 12.22 Aa	5.23 ± 0.56 Aa
	500 µg	22.70 ± 2.22 Aa	27.93 ± 5.43 Aa	5.23 ± 0.65 Aa
24 hours	Control	24.76 ± 0.07 Aa	15.71 ± 1.75 Aa	5.23 ± 0.56 Aa
	250 µg	25.69 ± 2.53 Ab	57.62 ± 9.60 Aa	7.85 ± 0.88 Aa
	500 µg	23.69 ± 1.35 Aa	26.18 ± 4.37 Aa	5.23 ± 0.77 Aa
48 hours	Control	25.78 ± 1.85 Ba	47.14 ± 7.86 Aa	5.23 ± 0.65 Aa
	250 µg	43.36 ± 5.63 Aa	49.75 ± 14.84 Aa	10.48 ± 1.75 Aa
	500 µg	28.56 ± 2.32 ABa	30.11 ± 5.68 Aa	5.23 ± 0.65 Aa
Treatments[4]		4.44 *	0.95 NS	5.83 *
Time		6.25 **	0.18 NS	0.83 NS
Treatments X Time		1.82 NS	0.39 NS	4.80 *
C.V.[4]		36.70	83.04	23.02

[1] Means (n = 8) followed by the same letter do not differ by the Tukey test (P <0.05).
[2] Analysis of statistical variance represented by capital letters compare in the column the different treatments within each experimental day, lowercase letters compare in the column the evolution of each treatment in different experimental days.
[3] Control (inoculated and untreated); Treated with 250 and 500 µg of zafirlukast/kg of body weight.
[4] CV- Coefficient of Variation (%); NS - Not significant; * significant for P <0.05; ** Significant for P <0.01
[5] ALT- Alanine aminotransferase; AST- Aspartate aminotransferase.

The serum values of total protein, albumin, cholesterol, triglycerides, glucose, and creatinine are shown in Table 3. The study of total protein in tilapia blood revealed a significant increase (p <0.01) in animals treated with 500 µg of zafirlukast when compared to tilapia treated with 250 µg in 6 HPI. The analyzes of albumin and glycemia did not show significant differences between treatments (Table 3). Circulating levels of triglycerides and cholesterol showed no significant difference between treatments, however there was a significant increase (p <0.05) in control fish over time (Table 3). The serum biochemical determination of creatinine in the blood of tilapia revealed a significant increase (p <0.05) in the control animals with a peak observed at 24 HPI, being even significantly higher when compared to the animals treated with 250 µg zafirlukast (Table 3).

Table 3 - Mean values[1] (± SE) and ANOVA[2] observed in the serum biochemical analysis of total protein, albumin, cholesterol, triglycerides, glycemia and creatinine in tilapia treated with zafirlukast and inoculated with *Aeromonas hydrophila* bacterins.

Period	Treatments [3]	Total Protein (g/dL)	Albumin (g/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Glycemia (mg/dL)	Creatinine (mg/dL)
6 hours	Control	2,92 ± 0.06 ABa	0.96 ± 0.05 Aa	62.54 ± 1.85 Ab	99.69 ± 8.18 Ab	54.66 ± 3.04 Aa	17.77 ± 5.28 Aab
	250 µg	2.76 ± 0.12 Ba	0.90 ± 0.06 Aa	67.37 ± 3.84 Ab	81.53 ± 4.78 Aa	44.00 ± 6.28 Aa	4.73 ± 0.33 Aa
	500 µg	3.37 ± 0.17 Aa	1.10 ± 0.04 Aa	93.89 ± 8.44 Aa	180.13 ± 38.96 Aa	53.16 ± 4.67 Aa	7.14 ± 0.66 Aa
24 hours	Control	3,00 ± 0.08 Aa	1.02 ± 0.05 Aa	115.25 ± 5.44 Aa	166.79 ± 27.67 Aab	60.16 ± 2.67 Aa	29.13 ± 8.16 Aa
	250 µg	2.88 ± 0.05 Aa	1.01 ± 0.04 Aa	96.28 ± 4.17 Aab	119.36 ± 5.73 Aa	42.50 ± 3.11 Aa	4.60 ± 0.45 Ba
	500 µg	3.03 ± 0.10 Aa	1.11 ± 0.02 Aa	117.98 ± 5.44 Aa	174.34 ± 17.51 Aa	53.00 ± 1.55 Aa	9.70 ± 3,11 ABa
48 hours	Control	3,45 ± 0.04 Aa	1.12 ± 0.05 Aa	116.33 ± 6.85 Aa	262.83 ± 54.05 Aa	44.50 ± 3.07 Aa	6.17 ± 0.81 Ab
	250 µg	3.12 ± 0.11 Aa	1.04 ± 0.06 Aa	110.79 ± 5.74 Aa	224.11 ± 18.12 Aa	38.80 ± 4.33 Aa	10.90 ± 2.00 Aa
	500 µg	3.03 ± 0.05 Aa	1.08 ± 0.03 Aa	94.60 ± 3.71 Aa	156.25 ± 14.29 Aa	41.80 ± 0.77 Aa	5.00 ± 0.58 Aa
Treatments[4]		2.95 NS	2.50 NS	1.85 NS	0.86 NS	3.50 *	5.44 **
Time		2.82 NS	1.93 NS	23.77 **	5.75 **	3.09 NS	1.94 NS
Treatments X Time		3.49 *	0.75 NS	4.91 **	2.42 NS	0.35 NS	2.77 *
C.V.[4] .		11.91	16.79	20.44	60.50	26.55	115.20

[1] Means (n = 8) followed by the same letter do not differ by the Tukey test (P <0.05).
[2] Analysis of statistical variance represented by capital letters compare in the column the different treatments within each experimental day, lowercase letters compare in the column the evolution of each treatment in different experimental days.
[3] Control (inoculated and untreated); Treated with 250 and 500 µg of zafirlukast/kg of body weight.
[4] CV- Coefficient of Variation (%); NS - Not significant; * significant for P <0.05; ** Significant for P <0.01

DISCUSSION

Tilapia treated with 500 µg zafirlukast showed polycythemia associated with microcytosis. These cases of microcytosis can occur in the course of inflammatory processes (Van Vranken, 2010). However, the side effect of treatment with 500 µg zafirlukast in fish during acute inflammation was evident. Several studies have demonstrated the hepatotoxicity of treatments with zafirlukast (Reinus et al., 2000; Scheen, 2001; Marcy et al., 2004). Such findings suggest the hypothesis that treatment with overdose (500 µg) has resulted in deleterious changes in tilapia.

Control fish showed an increase in blood glucose values 24 HPI, suggesting an increase in energy metabolism. These findings were associated with increased triglyceride and cholesterol values. The participation of steroid hormones such as cortisol during defense responses favor glucocorticoid actions by modulating neoglycogenic and glycogenolic mechanisms (Belo et al., 2005; 2012a). However, treatment with the anti-inflammatory zafirlukast controlled these glucocorticoid changes observed in control fish.

The serum evaluation of the enzymatic activity of AST and ALT represents an important indication of liver cytotoxicity or changes in the hepatocyte cell membrane permeability (Belo et al. 2012b). Tilapia treated with zafirlukast did not show changes in serum levels of AST and ALT in the first 48 hours. Such findings suggest the hypothesis of low hepatotocixity attributed to treatment with this leukotriene blocker in tilapia. Contrary to the findings of Reinus et al. (2000) and Actis et al. (2001) who reported severe liver changes associated with increased AST and ALT in humans undergoing prolonged treatment with zafirlukast. The low hepatotoxicity observed in tilapia may be the result of short-term treatment.

There was an increase in serum total protein levels in tilapias treated with 500 µg zafirlukast. These animals also showed an increase in erythrocyte cell counts with microcytosis. Taken together, these findings suggest the hypothesis of alterations in the liquid-electrolyte balance resulting from the overdose treatment with zafirlukast. Charlie-Silva et al. (2019) observed significant changes in circulating protein values during acute inflammatory reaction in tilapia after experimental infection with *A. hydrophila.*

According to Suja et al. (2004) and Shih et al. (2005), the functionality of liver tissues can be determined by the concentration of substances synthesized in the liver or belonging to specific metabolic processes, such as albumin, urea, glucose, cholesterol, triglycerides, and others. However, the results observed in this study did not reveal evidence of changes in liver functionality attributed to treatment with zafirlukast.

The increase in creatinine is an indicator of changes in renal functionality. Control tilapia showed a transitory increase in circulating creatinine values resulting from the acute inflammatory reaction. However, fish treated with zafirlukast presented normal serum creatinine levels. The decrease in plasma creatinine in mammals may be due to diuresis, in Nile tilapia this behavior can also be explained by the osmoregulatory control mechanism (Moraes et al., 2018), and the fish organism modulates diuresis to maintain the liquid-electrolyte balance and consequent the elimination of creatinine.

According to this experimental study realized in acute phase (48 hours post-treatment), it was possible to conclude that tilapia treated with 500 µg of zafirlukast showed adverse hematological effects characterized by polycythemia associated with microcytosis, and treatment with this leukotriene blocker did not show significant changes in cytotoxicity or liver and kidney function, demonstrating the clinical safety of zafirlukast treatment, especially when administered at a therapeutic dose of 250 µg.

ACKNOWLEDGMENTS

The authors would like to thank “The São Paulo Research Foundation-FAPESP” (process number: 2017/03094-7) for the financial support to carry out this research project.

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