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Trichlorfon-induced haematological and biochemical changes in *Cyprinus carpio*: ameliorative effect of propolis

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ABSTRACT: Trichlorfon is among the most commonly used products to treat fish parasites in aquaculture. We investigated the effectiveness of propolis in alleviating the toxicity of trichlorfon on haematological and oxidant/antioxidant parameters in carp *Cyprinus carpio*. Fish were exposed to sublethal concentrations (11 and 22 mg l^{-1}) of trichlorfon, and propolis (10 mg kg^{-1} of fish weight) was simultaneously administered. At the end of 14 d administration, blood and tissue (liver, kidney, gill) samples were collected. Haematological changes (red and white blood cell count, haemoglobin concentration, haematocrit level and erythrocyte indices: mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) were determined in the blood samples, while antioxidant parameters (malondialdehyde and reduced glutathione levels and superoxide dismutase, catalase and glutathione peroxidase activities) were evaluated in the liver, kidney and gill samples. Trichlorfon led to negative alterations in the haematological and antioxidant parameters investigated. The administration of propolis alleviated this effect and suggests that fish treated with trichlorfon improve their physiological status when fed a propolis-supplemented diet.

KEY WORDS: Organophosphate · Propolis · Haematology · Oxidative stress · Carp

INTRODUCTION

Extensive use of pesticides such as herbicides, insecticides and their mixtures in agriculture often leads to alterations in the chemical composition of natural aquatic systems, causing chronic toxicity to the freshwater fauna, particularly fish (Stara et al. 2012, Matviishyn et al. 2014). Organophosphate insecticides have been extensively used because they are highly effective and exhibit relatively non-persistent characteristics (Arufe et al. 2007, Mişe Yonar 2013, Mişe Yonar et al. 2014).

In intensive farming systems, the probability of parasite infestations is extremely high. Trichlorfon (dimethyl [2,2,2-trichloro-1-hydroxyethyl] phosphonate), an organophosphate compound, is one of the most commonly used products to treat against fish parasites in aquaculture. A wide range of concentrations of trichlorfon (0.25 to 25 g l^{-1}) and exposure times are used in the treatment of parasitic diseases caused by monogenetic trematodes and parasitic copepods (Chandrasekara & Pathiratne 2005, Guimarães et al. 2007). The recommended dose of trichlorfon to eradicate fish parasites varies from 0.1 to 1.0 mg l^{-1}, with 5 applications of 0.25 mg l^{-1}, weekly, indicated as the most effective treatment against fish parasitic infestation (Guimarães et al. 2007, Sinha et al. 2010). However, farmers often apply excessive doses of trichlorfon. The repeated use of this insecticide in extensive and intensive aquaculture constitutes a potential threat to non-targeted species such as fish, crabs, and shrimp (Guimarães et al. 2007,

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Sinha et al. 2010). In aqueous solution, trichlorfon is hydrolysed very quickly into dichlorvos, which is much more toxic and is a potent acetylcholinesterase inhibitor (Sinha et al. 2010). Dichlorvos is also used as a pesticide (Eraslan et al. 2010).

Propolis is a resinous substance collected by honeybees *Apis mellifera* from various tree buds, and bees use propolis for coating hive parts and also to seal cracks and crevices in the hive. Propolis composition is directly related to that of bud exudates from *Populus* spp., birch *Betula alba*, beech *Fagus sylvatica*, horse chestnut *Aesculus hippocastanum*, alder *Alnus glutinosa* and various conifers (Silici & Kutluca 2005). Its composition may vary according to geographic location and different plant sources, but crude propolis is composed basically of 45–50% resins and balsams, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% organic and mineral matter (Doğanyiğit et al. 2013). Flavonoids, aromatic acids, diterpenic acids and phenolic compounds appear to be the principal components responsible for the biological activities of propolis (Silici & Kutluca 2005). Of the biological activities of propolis, its antibacterial and antifungal activities are the most extensively investigated (Kujumgiev et al. 1999). Its pharmacological action, such as anticancer (Marcucci 1995), anti-inflammatory (Wang et al. 1993), antibiotic (Koo et al. 2000), antioxidative (Yonar et al. 2011, 2012, Selamoglu Talas et al. 2014), antiviral (Marcucci 1995), antifungal (Koc et al. 2005), anaesthetic, immunostimulant (Yonar et al. 2011), cytostatic effects (Ghisalberti 1979) and phagocytosis and haematological parameters (Çetin et al. 2010, Selamoglu Talas et al. 2012, Orun et al. 2013, Dotta et al. 2014), have been ascribed to the ethanolic extracts of propolis.

To date, the ameliorative role of propolis against trichlorfon-induced changes in the haematological parameters and oxidant/antioxidant status of fish has not been studied. Therefore, the objectives of the study were (1) to evaluate whether trichlorfon induces changes in the haematological parameters and the oxidant/antioxidant status of the blood and tissues of carp and (2) to evaluate the role of propolis in alleviating the negative effects of trichlorfon.

MATERIALS AND METHODS

Propolis

The propolis used in the present study was poplar *Populus nigra* L. propolis collected manually from colonies of honeybee *Apis mellifera caucasica* in the vicinity of Kayseri Province, Central Anatolia (Turkey). Ethanol extracts from the propolis sample were used throughout this study. The propolis doses were maintained in a dark environment in a deep freezer (–20°C) until processing. Propolis powder (30 g) was dissolved in 100 ml of 70% ethanol solution for 1 wk at room temperature. After 1 wk, the ethanol extract was filtered and then evaporated using a vacuum evaporator (Bankova et al. 2002). The chemical content of propolis used in this study had previously been identified by gas chromatography/mass spectrophotometry (GC/MS) (Yonar et al. 2011), and the main compounds are presented in Table 1.

Chemicals

The commercial product trichlorfon (trade name: Neguvon, Bayer), available at a concentration of 75% (w/v), was used in this study. Different dilutions were prepared by adding water. All other chemicals were purchased from Sigma-Aldrich Chemical.

Fish

Carp *Cyprinus carpio* of both sexes (mean ± SD weight 46.3 ± 7.7 g) were obtained from local fish

Table 1. Chemical composition of the ethanolic extract of propolis from poplar *Populus nigra* L., assessed by gas chromatography/mass spectrophotometry (data from Yonar et al. 2011). RT: retention time (minutes) (m/z = 219); IC: ion current (%). NB IC generated depends on the characteristics of the compounds concerned and is not a true quantitation

Compound	RT	IC
Phenolic compounds		
Chrysin	52.64	6.41
2-methoxy-4-vinylphenol	12.99	1.19
4-vinylphenol	10.34	0.43
Aliphatic, aromatic and fatty acids		
Benzoic acid	9.09	0.43
Ferulic acid	41.33	2.26
9-octadecenoic acid	38.97	0.28
Esters		
Benzyl cinnamate	36.98	3.00
Terpenes		
Beta-eudesmol	24.08	0.77
Aldehydes, ketones and others		
Benzeneethanol	8.42	0.26
Chrysophanol	52.28	20.64
Benzaldehyde	14.90	0.41
Docosane	44.35	0.40
2-propen-1-one	45.44	11.28
4H-1-benzopyran-4-one	47.46	15.06
2,4-cycloheptadien-1-one	54.05	7.25

ponds (Elazig, Turkey). Prior to the test, fish were acclimated to laboratory conditions for 2 wk in a 1500 l tank under natural photoperiod in aerated and dechlorinated tap water. Water parameters measured with Thermo Scientific Orion 5-Star were 17 ± 2°C, 8.0 ± 0.5 mg l^{-1} O_2 and pH 7.6 ± 0.4. During this period, the fish were fed commercial fish food twice daily (36% crude protein).

Feed preparation and experimental setup

For experimental groups of carp, a commercial basal diet was crushed and mixed with 10 mg of propolis per kg of fish. Feed was transformed into pellets, dried and stored at +4°C.

The experiments were carried out in 96 l (80 × 40 × 30 cm) glass aquaria under the same water conditions as above for a duration of 14 d. The fish were divided into 6 groups as follows:

- Group 1 (C), the control group, was maintained in tap water and fed commercial basal diet without propolis.
- Group 2 (P) was maintained in tap water and fed propolis-supplemented diet.
- Group 3 (T1) was exposed to 11 mg l^{-1} trichlorfon and fed commercial basal diet without propolis.
- Group 4 (T1+P) was exposed to 11 mg l^{-1} trichlorfon and fed propolis-supplemented diet.
- Group 5 (T2) was exposed to 22 mg l^{-1} trichlorfon and fed commercial basal diet without propolis.
- Group 6 (T2+P) was exposed to 22 mg l^{-1} trichlorfon and fed propolis-supplemented diet.

The entire experiment was independently repeated, and each replicate of each group contained 5 fish, totaling 60 fish. The sublethal concentrations of trichlorfon (11 and 22 mg l^{-1}) were selected on the basis of a study by Pala (2013), who showed that the 96 h LC_{50} for trichlorfon was 61.98 mg l^{-1}. The experimental aquaria were aerated, and the test media were replaced every day.

Haematological and biochemical analysis

At the end of the experiment, fish were anaesthetised with benzocaine (25 mg l^{-1}), and blood samples were drawn from the caudal peduncle using a medical syringe with EDTA as an anticoagulant. The blood was used immediately for haematological analysis. The red blood cell (RBC) and white blood cell (WBC) counts were made using a haemocytometer and Natt & Herrick (1952) solution. The haemoglobin concentration (Hb) was determined with Drabkin's reagent read at 540 nm (Drabkin 1946), and the haematocrit (Ht) was determined by a microhaematocrit centrifugation technique. The erythrocyte indices (mean corpuscular volume, MCV; mean corpuscular haemoglobin, MCH; and mean corpuscular haemoglobin concentration, MCHC) were calculated by standard formulas with the data of the Ht, RBC and Hb (Saglam et al. 2003, Saglam & Yonar 2009).

Immediately after the blood samples were collected, the liver, kidney, and gill were carefully removed, washed in ice cold physiological saline, and stored at −40°C until the biochemical assays, which were performed within 1 mo after extraction. The tissue was homogenised in a Teflon-glass homogeniser in buffer containing 1.15% KCl at a 1:10 (w/v) ratio to the whole homogenate. The homogenate was centrifuged at 18 000 × *g* (4°C for 30 min) before the determination of the malondialdehyde (MDA) and reduced glutathione (GSH) levels and the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities. The method described by Placer et al. (1966) was used to determine the MDA levels in all tissues. The measurements were performed in accordance with the method described by Sun et al. (1988) for determination of the tissue SOD activity. The CAT activity measurements were performed in accordance with the method described by Aebi (1983). The GSH-Px activity measurements were performed following Beutler (1975). The GSH level was measured through the dithionitrobenzoic acid recycling method described by Ellman (1959). The protein levels in the tissues were determined after Lowry et al. (1951).

Statistical analyses

The statistical significance of the differences between the data obtained from the control and that obtained from the experimental groups was analysed via 1-way ANOVA and Duncan's *post hoc* test using SPSS 21.

RESULTS

Fish behaviour during the experiment

During the experiment, the control and experimental carp showed normal feeding behaviour. Furthermore, there were no signs of respiratory distress such

as rapid ventilation, increased rate of gill cover movements or floating at the surface of the water. There were no mortalities.

Haematological and biochemical analysis

Trichlorfon alone caused a significant decrease in the RBC and WBC count, Ht level, Hb concentration and erythrocyte indices (Table 2). Simultaneous treatment with propolis provided a marked normalisation of the haematological parameters compared with the trichlorfon groups. In comparison to the control group, the WBC count was significantly higher in the group administered propolis alone. No significant difference in the other haematological parameters was observed between fish fed propolis and the control group.

The MDA level was significantly increased in the liver, kidney and gill samples of the groups treated only with trichlorfon. Treatment with propolis resulted in a decrease in the tissue MDA levels compared with the trichlorfon-treated groups, but they were still higher than that of the control. Treated fish fed propolis presented no significant difference in the tissue MDA levels compared with those of the control group (Table 3).

Significant decreases in the activities of SOD, CAT and GSH-Px were observed in the trichlorfon-treated fish when compared to non-treated ones. Co-treatment with propolis provided a marked normalisation of the tissue SOD, CAT and GSH-Px activities. There were no significant differences between enzyme activities of the group that was administered propolis alone and the control group (Table 3).

The GSH level was significantly decreased in the trichlorfon-treated group compared to the control group. The trichlorfon-treated fish fed propolis had higher GSH levels than the trichlorfon groups and the control group. Fish fed propolis exhibited no significant difference in the tissue GSH levels compared to the control group (Table 3).

DISCUSSION

The evaluation of haematological characteristics in fish has become an important means of understanding normal and pathological processes and toxicological impacts (Li et al. 2011, Dotta et al. 2014). Haematological parameters, such as the RBC counts, Hb concentrations, Ht levels and the erythrocyte indices (MCV, MCH and MCHC), are widely used to evaluate the toxic stress that is induced by environmental contaminants (Saravanan et al. 2011). In this study, the RBC, Ht and Hb levels, and the erythrocyte indices were decreased significantly in the trichlorfon-treated fish. Decreases in the RBC count and the Ht and Hb levels may be an indicator of anaemia due to the inhibition of erythropoiesis, haemosynthesis or osmoregulatory dysfunction or to an increased rate of erythrocyte destruction in the haematopoietic organ (Vani et al. 2011, Mişe Yonar et al. 2014). In addition, decreases in the MCV, MCH and MCHC values indicate microcytic hypochromic anaemia (Prusty et al. 2011). Unlike mammals, the haematopoietic system of fish is mainly located in the interstitium of the kidney. Thus, a reduction in the haematological parameters may be attributed to the malfunctioning of the haematopoietic system caused by morphological alterations in the renal interstitium (Li et al. 2011). However, the haematological values of treated fish fed propolis were similar to those of non-treated fish fed without propolis and were significantly different from the trichlorfon-treated fish. The findings of our study suggest that propolis reduces the harmful effects of trichlorfon by

Table 2. Effect of trichlorfon, propolis and their combination on some haematological parameters in the control and experimental groups of carp *Cyprinus carpio*. C: control, P: propolis (10 mg kg^{-1} fish d^{-1}), T1: trichlorfon (11 mg l^{-1}), T1+P: trichlorfon (11 mg l^{-1}) plus propolis (10 mg kg^{-1} fish d^{-1}), T2: trichlorfon (22 mg l^{-1}), T2+P: trichlorfon (22 mg l^{-1}) plus propolis (10 mg kg^{-1} fish d^{-1}). RBC: red blood cell (erythrocyte) counts, WBC: white blood cell (leukocyte) counts, Ht: haematocrit, Hb: haemoglobin concentration, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration. Different superscripts in the same column indicate significant differences between groups ($p < 0.05$). Values are means ± SE

Group	RBC ($\times 10^6$)	WBC ($\times 10^3$)	Ht (%)	Hb (g dl^{-1})	MCV (μm^3)	MCH (pg)	MCHC (%)
C	1.51 ± 0.14^{d}	41.08 ± 3.11^{b}	36.28 ± 5.13^{c}	8.14 ± 0.92^{b}	241.49 ± 38.09^{d}	54.72 ± 4.11^{a}	23.36 ± 3.17^{a}
P	1.50 ± 0.11^{d}	46.22 ± 5.27^{c}	35.59 ± 4.88^{c}	8.37 ± 0.69^{b}	239.67 ± 33.57^{d}	55.80 ± 3.89^{a}	22.90 ± 3.59^{a}
T1	1.29 ± 0.13^{a}	25.03 ± 7.19^{a}	27.13 ± 6.29^{b}	4.73 ± 0.71^{a}	208.00 ± 37.75^{b}	38.77 ± 6.27^{b}	17.45 ± 4.20^{b}
T1+P	1.52 ± 0.17^{d}	42.20 ± 6.38^{b}	34.80 ± 5.32^{c}	7.92 ± 0.89^{b}	230.84 ± 29.36^{c}	51.05 ± 5.26^{a}	22.95 ± 4.16^{a}
T2	1.21 ± 0.15^{b}	24.67 ± 3.26^{a}	23.39 ± 5.49^{a}	4.59 ± 0.80^{a}	191.57 ± 28.05^{a}	36.38 ± 6.33^{b}	18.37 ± 3.22^{b}
T2+P	1.47 ± 0.16^{c}	40.82 ± 5.17^{b}	33.66 ± 6.01^{c}	7.80 ± 1.03^{b}	226.97 ± 29.91^{c}	51.79 ± 4.72^{a}	23.29 ± 3.46^{a}

Table 3. Malondialdehyde (MDA) and reduced glutathione (GSH) levels and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities in the control and experimental groups of carp *Cyprinus carpio* (see Table 2 for groups). k: first-order rate constant. Per tissue, different superscripts within a column indicate significant differences between groups ($p < 0.05$). Values are means ± SE

Group	MDA (nmol mg^{-1} protein)	SOD (U mg^{-1} protein)	CAT (k mg^{-1} protein)	GSH-Px (U mg^{-1} protein)	GSH (µmol mg^{-1} protein)
Liver					
C	0.81 ± 0.05^{a}	2.76 ± 0.41^{a}	4.02 ± 0.88^{d}	2.44 ± 0.53^{b}	81.24 ± 7.14^{c}
P	0.73 ± 0.03^{a}	2.94 ± 0.37^{a}	4.21 ± 1.10^{d}	2.62 ± 0.60^{b}	82.35 ± 8.00^{c}
T1	1.92 ± 0.09^{c}	1.42 ± 0.59^{c}	2.23 ± 0.97^{b}	1.70 ± 0.71^{a}	36.93 ± 11.37^{b}
T1+P	1.16 ± 0.06^{b}	2.37 ± 0.66^{b}	3.38 ± 0.73^{c}	2.79 ± 0.83^{b}	84.02 ± 10.26^{c}
T2	2.25 ± 0.04^{d}	1.29 ± 0.72^{c}	1.91 ± 0.92^{a}	1.51 ± 0.58^{a}	29.18 ± 8.17^{a}
T2+P	1.23 ± 0.07^{b}	2.88 ± 0.56^{a}	3.20 ± 1.31^{c}	3.30 ± 1.13^{c}	91.44 ± 9.10^{d}
Kidney					
C	1.27 ± 0.35^{a}	2.35 ± 0.43^{c}	1.80 ± 0.57^{c}	1.45 ± 0.37^{c}	15.97 ± 2.13^{b}
P	1.22 ± 0.46^{a}	2.42 ± 0.57^{c}	1.89 ± 0.63^{c}	1.42 ± 0.42^{c}	17.20 ± 2.42^{b}
T1	2.17 ± 0.68^{c}	1.16 ± 0.84^{a}	1.14 ± 0.82^{a}	0.76 ± 0.61^{a}	8.30 ± 2.76^{a}
T1+P	1.44 ± 0.57^{b}	2.59 ± 0.68^{c}	1.68 ± 0.76^{b}	1.41 ± 0.76^{c}	21.04 ± 4.83^{c}
T2	2.48 ± 0.87^{d}	1.10 ± 0.71^{a}	1.02 ± 0.55^{a}	0.70 ± 0.58^{a}	6.75 ± 2.95^{a}
T2+P	1.51 ± 0.55^{b}	2.04 ± 0.80^{b}	1.91 ± 0.91^{c}	1.30 ± 0.82^{b}	26.06 ± 6.79^{d}
Gill					
C	0.86 ± 0.46^{a}	1.55 ± 0.33^{b}	1.36 ± 0.34^{d}	1.20 ± 0.41^{c}	8.32 ± 1.89^{b}
P	0.78 ± 0.35^{a}	1.52 ± 0.49^{b}	1.40 ± 0.42^{d}	1.18 ± 0.30^{c}	8.51 ± 2.51^{b}
T1	2.04 ± 0.61^{c}	0.83 ± 0.28^{a}	0.87 ± 0.61^{a}	0.95 ± 0.53^{a}	5.93 ± 2.17^{a}
T1+P	1.49 ± 0.39^{b}	1.80 ± 0.73^{c}	1.21 ± 0.59^{c}	1.12 ± 0.68^{c}	18.47 ± 5.26^{c}
T2	2.96 ± 0.44^{d}	0.72 ± 0.19^{a}	0.70 ± 0.34^{b}	0.81 ± 0.51^{b}	4.89 ± 1.90^{a}
T2+P	1.52 ± 0.29^{b}	1.49 ± 0.68^{b}	1.16 ± 0.22^{c}	1.38 ± 0.76^{d}	20.71 ± 4.86^{c}

maintaining optimal haematological values. Leucocytes play an important role in nonspecific or innate immunity, and the leucocyte count/activity indicates the health status of a fish (Secombes 1996). Leucocytes are involved in the control of immunological functions, and changes in WBC counts after exposure to toxicants indicate a decrease in the nonspecific immunity of the fish (Saravanan et al. 2011); the WBC count was significantly lower in the trichlorfon-treated fish. Similar changes in the WBC count in common carp have also been reported by other authors. Chandrasekara & Pathiratne (2005) reported a decrease in the WBC count of common carp after trichlorfon exposure. Dunier et al. (1991) reported a strong immunodepressive effect of trichlorfon on 2 major functions of cellular immunity in *in vitro* assays for lymphocyte proliferation and myeloid cell respiratory burst activities. Consistent with previous investigations (Chandrasekara & Pathiratne 2005, Dunier et al. 1991), this decrease in the WBC count may be related to trichlorfon-induced leucopaenia. The present study suggests that propolis restores the leucopaenia induced by trichlorfon in carp.

Oxidative stress is an imbalance between production of free radicals and reactive metabolites, and their elimination by protective antioxidants (Piner & Üner 2013). Oxidative damage is related to the formation of reactive oxygen species (ROS) and can occur when the antioxidant and detoxifying systems are deficient and not able to neutralise the active intermediates produced by xenobiotics and their metabolites (Mişe Yonar 2013). Many classes of environmental pollutants or their metabolites exert toxicity related to oxidative stress and can cause oxidative damage in fish (Stara et al. 2012).

Lipid peroxidation is an indicator of the oxidative damage of cellular components (Ferreira et al. 2005). MDA as a main end product of lipid peroxidation is one of the indices of lipid peroxidation (Halliwell 1994, Yonar & Mişe Yonar 2010). In the present study, significant increase in the MDA levels in all tissues was observed in the trichlorfon-treated fish compared to non-treated fish, corroborating previous studies. For example, trichlorfon in Nile tilapia *Oreochromis niloticus* (Thomaz et al. 2009) was found to induce oxidative stress, and as did dichlorvos hydrolysed from trichlorfon in carp, catfish *Ictalurus nebulosus* (Hai et al. 1997) and European eel *Anguilla anguilla* (Peña-Llopis et al. 2003). These increases in MDA can most likely be ascribed to an excessive production of ROS. However, the simultaneous treatment with propolis decreased the levels of MDA in the different tissues of trichlorfon-treated fish, which may be due to an antiradical effect related to the

phenolic compounds found in propolis, the radical scavenging of which is well documented (Yonar et al. 2011, 2012).

The first line of defence against oxidative stress consists of the antioxidant enzymes SOD, CAT and GSH-Px, which convert superoxide anions (O_2^-) into H_2O_2 and then into H_2O and O_2 (Stara et al. 2012). In the present study, we observed a significant decrease in the SOD, CAT and GSH-Px activity in fish treated with trichlorfon alone when compared to untreated fish. Similar results were reported by Thomaz et al. (2009), who evaluated the effects of trichlorfon on *O. niloticus*. They documented significant decreases of SOD and CAT activity in liver and heart. This decrease in the SOD activity may be the result of excessive free radical production, such as the superoxide anion and hydrogen peroxide, direct damage of its protein structure by pesticide or a direct action of pesticide on the synthesis of the enzyme. Propolis administration increased the SOD, CAT and GSH-Px tissue activity of trichlorfon-treated fish; this increase can be attributed to the inhibition of superoxide radical formation or free radical scavenging activity of propolis.

GSH has the ability to protect cells from oxidative stress and plays a critical role in detoxification reactions by acting both as a nucleophilic scavenger of various undesirable compounds and their toxic metabolites and as a specific substrate for the GSH-dependent enzymes (Sk & Bhattacharya 2006). GSH also acts as a reactant in conjunction with electrophilic substances. Thus, GSH levels are an indicator of the detoxification ability of an organism (Stara et al. 2012). In the present study, a significant decrease in the GSH levels in all tissues was observed in the trichlorfon-treated fish compared to the control group. Similarly, Monteiro et al. (2009) demonstrated decreases in GSH levels in liver, gill and white muscle of the freshwater characid fish matrinxã *Brycon cephalus* after exposure to methyl parathion. Different results were obtained by Thomaz et al. (2009) for specimens of *O. niloticus* exposed to a sublethal concentration (0.5 mg l^{-1}) of trichlorfon for 96 h. They reported that trichlorfon exposure significantly increased GSH level in gills and liver. The overproduction of ROS, as indicated by the high level of lipid peroxidation, may be associated with the depletion of GSH. The depletion in the present study is one of the factors responsible for the enhanced lipid peroxidation (Monteiro et al. 2009).

This study shows that fish treated with trichlorfon against parasites could improve their physiological status when fed a propolis-supplemented diet.

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