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PII: S1532-0456(19)30399-0

DOI: https://doi.org/10.1016/j.cbpc.2019.108683

Reference: CBC 108683

To appear in: *Comparative Biochemistry and Physiology, Part C*

Received date: 1 October 2019

Revised date: 15 December 2019

Accepted date: 18 December 2019

Please cite this article as: P.R. de Oliveira dos Santos, M.J. Costa, A.C.A.D. Santos, et al., Neurotoxic and respiratory effects of human use drugs on a Neotropical fish species, Phalloceros harpagos, *Comparative Biochemistry and Physiology, Part C*(2019), https://doi.org/10.1016/j.cbpc.2019.108683

Short communication

Neurotoxic and respiratory effects of human use drugs on a Neotropical fish species, *Phalloceros harpagos*.

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ABSTRACT

Pharmaceutical drugs are usually and continuously carried to aquatic environment in different ways. Thus, they are pseudo-persistent in the environment, and they may exert deleterious effects on aquatic organisms. The objective of the present study was to investigate the acute and chronic effects of two widely used pharmaceutical drugs, paracetamol (analgesic and antipyretic) and propranolol (β-blocker) on the activity of specific biomarkers (namely cholinesterase enzymes and lactate dehydrogenase) of the neotropical fish *Phalloceros harpagos*. The results obtained indicate an inhibition of the activity of the enzyme lactate dehydrogenase (LDH) after acute exposure to paracetamol, and an increase in cholinesterase activity in acutely propranolol-exposed fishes. Chronic exposure to both drugs did not modify the enzymatic activities. Such short-term changes in enzymatic activities may be harmful to organisms, altering the preferential pathway of energy metabolism, and may induce behavioral changes that may compromise prey capture and predator escape, and in the longer term may induce population declines.

Introduction

The great number of drugs produced by pharmaceutical industry, and the implementation of healthcare assistance provided to human populations, led to a progressive consumption of such chemicals and to the increase of their presence in aquatic environments (ALTÝNDAG *et al*., 2008; FENT, 2008; BRANDÃO *et al*., 2013; NUNES *et al*., 2015; PEREIRA *et al.*, 2018). Pharmaceutical drugs can enter the environment by means of animal and human excretions containing their residues, and the inefficient degradation of domestic effluents at sewage treatment plants. Generally, due to their slightly lipophilic nature, these compounds are readily absorbed by non-target organisms (NUNES *et al*., 2014). The target receptors that are activated by drugs are evolutionarily conserved in both vertebrates and invertebrates, resulting in similar effects (BRANDÃO *et al*., 2013). These drugs exert biological activity at low concentrations and, being pseudo-persistent in the environment, may exert long-term deleterious effects on aquatic organisms, which are still poorly characterized in animals from tropical ecosystems (DAUGHTON, 2002; OLIVEIRA *et al*., 2015; EBELE *et al*., 2017).

Propranolol is a β-blocking drug used for human therapy for decades (Alder et al., 2010), that acts by blocking the effects of physiological agonists on β-adrenergic receptors and is commonly used for the treatment of circulatory/cardiac pathologies (OWEN *et al*., 2007). Given its use, propranolol is highly disseminated in the aquatic ecosystem. According to Roberts and Thomas (2006), the presence of propranolol was detected in all samples collected at the Tyne River estuary, UK, in levels that ranged from 35 to 107 ng l^{-1}. Propranolol was also found in the Santa Ana River, in the United States of America, according to Fono and Sedlak (2005). In the Ebro River, Spain, propranolol was also detected in levels up to 63ng L^{-1}, as shown by Gros et al. (2007). Levels of propranolol measured in German river reached 590 ng L^{-1}, according to Ternes (2001). Despite the wide environmental presence of propranolol, its acute effects only occur at much higher levels of exposure. The 96-h LC_{50} of propranolol in the zebrafish larvae was 2.48 mg/L, according to Sun et al. (2014). According to Kim et al. (2009), the 24-hLC50 of this drug for the freshwater crustacean *Thamnocephalus platyurus* is of 10.31 mgl^{-1}, while for the fish species *Oryzias latipes* the calculated 96h-LC_{50} was of 11.40 mgl^{-1}.

Paracetamol, a widely used analgesic and antipyretic drug, presents inhibitory action on prostaglandin biosynthesis (BOUTAUD *et al*., 2002). Paracetamol, given its wide use, had been found in very diverse locations, such as in European effluents, in levels reaching 6 μg L^{-}

[1] (TERNES, 1998), 10 μg L^{-1} in surface waters in the United States of America (KOLPIN et al., 2002), 65 μg L^{-1} in the Tyne River in the UK (ROBERTS & THOMAS, 2006), and 30,421 ng L^{-1} in São Carlos, São Paulo, Brazil (CAMPANHA et al., 2014). Concentrations of 0.06-0.119 μg L-1have already been detected in the environment (ROBERTS and THOMAS, 2006). The already documented toxicity of paracetamol towards aquatic organisms shows that environmental levels are far from causing lethal effects. In fact, and according to Kim et. (2007), the 48h- and 96h-LC_{50} values for the microcrustacean *Daphnia magna* were of 30.1 and 26.6 mg l^{-1}, respectively; the same parameters, but calculated for *O. latipes*, exceeded the highest tested concentrations, of 160 mg l^{-1}. Paracetamol metabolism results always in the production of a bioactive intermediate, N-acetyl-p-benzoquinoemine (NAPQI) that is however conjugated with reduced glutathione and excreted. However, in high amounts, the production of NAPQI exceeds the amount of available intracellular GSH, and NAPQI accumulates, causing oxidative stress and inducing the activation of defensive antioxidant responses (XU *et al*., 2008; PEREIRA *et al*., 2018). If not neutralized and adequately excreted, this activated metabolite can modify macromolecules and cause tissue damage due to oxidative stress (XU *et al*., 2008; NUNES, 2008; BRANDÃO *et al*., 2011).

Acetylcholinesterase is a postsynaptic enzyme that hydrolyses the neurotransmitter acetylcholine, so that the neuron returns to its resting state upon activation (ARAÚJO *et al.*, 2016) avoiding excessive cholinergic transmission (RAMOS *et al*., 2012). This enzyme acts both in the cholinergic synapses of the central and peripheral nervous systems, being an early and sensitive biomarker of exposure to many environmental contaminants, also presenting low cost and good reproducibility (NUNES, 2011). Propranolol is considered a competitive inhibitor of ChEs (ALKONDON et al., 1986; STANKOV-JOVANOVIĆ et al., 2012). The study conducted by Dash et al. (1990) showed that propranolol could significantly inhibit the acetylcholinesterase activities of brain and heart tissues of rodents chronically exposed to this substance. Propranolol was also shown be involved in cholinesterasic inhibition, in mussels as demonstrated by Amiard and Amiard-Triquet (2013). In addition, similar data obtained by Ek et al., (2019) evidenced that propranolol exposure could elicit anticholinesterasic effects on the *Gammarus* spp, suggesting that propranolol may be an acetylcholinesterase inhibitor by unknown mechanisms of toxic action. Cholinesterases (ChEs) inhibition is sometimes followed by behavioral changes, namely in rats (PADILLA, 1995), which may imply an alteration of patterns of reproduction, migration, capture of prey and predator avoidance (NUNES, 2011).

The enzyme lactate dehydrogenase (LDH) acts in the glycolytic pathway, being responsible for the regeneration of NAD^+ during anaerobic glycolysis through the reduction of pyruvate to lactate, producing ATP. LDH can be activated in organisms under stressful conditions, such as low oxygen levels or when exposed to toxic agents (WU & LAN, 1997). Changes in its activity may be triggered by chemical substances, and modification of its levels signal alterations in respiration patterns of metabolically active tissues (NUNES, 2010).

The objective of the present study was to investigate the acute and chronic effects of two widely used pharmaceutical drugs, paracetamol (analgesic and antipyretic) and propranolol (β-blocker) on the activity of specific biomarkers (namely the enzymes acetylcholinesterase (PEREIRA et al,. 2019) and lactate dehydrogenase) of the neotropical fish *Phalloceros harpagos*.

Methods

The poecilide *Phalloceros harpagos* corresponds to a widely distributed fish species that occurs in the basin of the Paraná-Paraguay River in South America, and in its coastal basins, in shallow waters, being a representative species of the neotropical area (LUCINDA, 2007; SOUZA *et al*., 2009). Poecilids (including *P. harpagos*) are characterized by their small size, sexual dimorphism and high fecundity through viviparous or ovoviviparous strategies (MAZZONI *et al*., 2002). This species presents favorable features for its laboratory use, such as high sensitivity to environmental contaminants, availability and abundance in its habitat, easy capture and adaptation to laboratory conditions, being representative of the neotropical environment. These features turn it into a good candidate for ecotoxicological studies (COSTA *et al*., 2008; PEREIRA *et al*., 2018; MATUS *et al*., 2018).

Immediately after being manually captured in a stream at an unpolluted location (for more details, please check the study by Pereira et al., 2018), test organisms were kept under acclimation and quarantine conditions, similar to those of the both exposure modalities (acute and chronic) for never less than 3 weeks, during which were fed *ad libitum*. In addition, fish were fed the day before the onset of both experiments.

Acute fish exposures were based on OECD 203 guideline (1995), with modifications (for details, see PEREIRA *et al*., 2018). The animals were exposed in 2L polyethylene bottles (previously used for human water consumption), filled with 250 ml Klarina® mineral water. The fish were exposed individually, i.e. each vessel contained only one organism. Thus, the experimental design for both exposures was of 15 specimens (replicates, fish individually

exposed), for each concentration tested, and control. Therefore, six experimental groups (control and 5 different concentrations of each drug) were adopted. The concentrations of paracetamol were 0 (control group), $5.29x10^{-8}$, $5.29x10^{-7}$, $5.29x10^{-6}$, $5.29x10^{-5}$ and $5.29x10^{-4}$ M. The concentrations of propranolol were 0 (control group), $3.380x10^{-10}$, $3.380x10^{-9}$, $3.380x10^{-8}$, $3.380x10^{-7}$ and $3.380x10^{-6}$ M. The lowest concentrations used for exposure were selected based on those already reported to occur in the environment (TERNES, 1998; KOLPIN *et al*., 2002; ROBERTS and THOMAS, 2006; CAMPANHA *et al*., 2015). Fish were exposed for a total of 96h, and 48h after the onset of the exposure, all media were renewed, and drug levels were re-established. Abiotic parameters, namely conductivity, temperature, oxygen concentration and media pH, were measured every day, for test validation purposes.

Chronic fish exposures were based on OECD 215 (2000) guideline with modifications (for details, see PEREIRA *et al*., 2018). Fish were exposed individually (1 fish per bottle, similarly to what was adopted for acute exposures) for 28 days. Five different concentrations of paracetamol were selected, 0 (control), $3.3077x10^{-8}$, $6.61x10^{-8}$, $1.323x10^{-7}$, $2.646x10^{-7}$ and $5.29x10^{-7}$ M. This set of concentrations was based on those already reported for this drug in the aquatic compartment (UTRILLA *et al*., 2013; COETSIER *et al.*, 2009; PEREIRA *et al*., 2018; THOMAS *et al*., 2014). The same procedure was used to define the concentrations of propranolol: 0 (control), $2.112x10^{-10}$, $4.225x10^{-10}$, $8.45x10^{-10}$, $1.69x10^{-9}$, and $3.380x10^{-9}$ M. Abiotic parameters were measured, similarly to what was done for acute exposures.

After the two exposure periods (acute and chronic), the fish were euthanized by hypothermia (water with ice, until they lost the posture reflex) and decapitation. Dissection was performed on Petri dishes on ice. The dorsal muscle tissues were isolated and put immediately in buffer specific for each biochemical analysis.

The biological material for the determination of lactate dehydrogenase (LDH) enzymatic activity was homogenized in 500 µL of Tris/NaCl buffer solution, pH = 7.2 at 4°C. Samples for determination of acetylcholinesterase activity (AChE) were homogenized in 500 µL of 0.1 M phosphate buffer solution, pH = 7.2. The homogenization was performed using a Ika T-10 basic Ultra-Turrax equipment, maintaining the microtube at 4°C. The homogenized samples were centrifuged at 4°C for 3 minutes at 3300g (centrifuge Hettich zentrifugen 320 R). After centrifugation, the supernatants were collected in Eppendorf microtubes and stored at -80°C until the assays were performed. The quantification of cholinesterase activity involved the method described by Ellman (1961), based on the hydrolysis of the substrate

acetylthiocholine (synthetic structural isomer of the neurotransmitter acetylcholine) by acetylcholinesterase, resulting in acetate and thiocoline. This product complexes with dithiobisnitrobenzonate (DTNB), resulting in a yellowish colored compound, the formation of which can be determined at 412 nm. The assays were performed with the Synergy HTX reader multi-mode reader, Bio-tek microplate reader. The results were expressed as nmol per minute per mg of protein.

This assay was based on the reduction of the pyruvate (substrate) and the simultaneous oxidation of β-NADH due to the presence of LDH (VASSAULT, 1983). Absorbance values were monitored at 340nm over time, and activity was calculated by recording the decrease of the absorbance value, according to the method described by Vassault (1983). The activity results were expressed as nmol per minute per mg of protein.

Quantification of the total soluble protein in samples was performed using the method of Bradford (1976) to express the result of the enzymatic activity as a function of the protein content. Protein quantification was based on the binding of the Bradford reagent (1976) to the total protein, resulting in a stable color complex whose absorbance was quantified at 595 nm. The protein standards were bovine γ-globulin with a concentration of 1 mg. mL^{-1}

Statistical analysis was performed using GraphPad Prism 6.0 software. Normal distributions of averages (normality test, Shapiro-Wilk), and homogeneity of variances (Bartelet test) were verified. If the normal distributions occurred, the data were analyzed by ANOVA followed by the Dunnet test. In the case of non-parametric results, the data were analyzed by the Kruskal-Wallis and Dunn tests. The significance level adopted for the statistical test was 0.05.

Results

Acute exposure to paracetamol did not induce a significant alteration in terms of cholinesterase activity ($F_{5,79}$ = 0.8110; p = 0.5453) evaluated in muscle tissue of *P. harpagos* (Fig. 1a), whereas for muscle lactate dehydrogenase activity there were significant differences ($F_{5.82}$ = 14.83, p <0.0001), namely an inhibition of the enzymatic activity in all concentrations of this drug (Fig. 2a).

Acute exposure to propranolol induced no significant differences for lactate dehydrogenase activity ($F_{5,78}$ = 1.594; p = 0.1716) in *P. harpagos* muscle tissue (Fig. 2b). However, cholinesterase activity ($F_{5,72}$ = 6.293, p = <0.0001) was significantly increased, specifically for organisms exposed to concentrations of $3.380x10^{-9}$ M and $3.380x10^{-6}$ M (Fig. 1b).

There were no significant differences in the activities of cholinesterase enzymes ($F_{5,80}$ = 1.184, p = 0.3244) nor of lactate dehydrogenase ($F_{5,77}$ = 1.296, p = 0.2724) in animals chronically exposed to paracetamol (Fig. 1c and Fig. 2c).

There were no significant differences in the enzymatic activities of cholinesterases ($F_{5,68}$ = 2.118; p = 0.0656) nor of lactate dehydrogenase ($F_{5,78}$ = 2.024; p = 0.0843) measured in animals chronically exposed to propranolol (Fig. 1d and Fig. 2d).

Discussion

The results obtained indicated an inhibition of the activity of the enzyme lactate dehydrogenase (LDH) after acute exposure to paracetamol. The decrease in lactate dehydrogenase activity observed in the present study may have occurred because the organism may have favored the use of the aerobic route for energy production (NUNES *et al*., 2015), suggesting that the energy balance of aerobic processes was enough to supply the metabolism of the fish under study. This suggestion is supported by the study conducted by Matus *et al.* (2018), who analyzed the behavior, tegumentary staining and glycogen reserves in the liver of *P. harpagos* during acute exposure to the same concentrations of paracetamol. Paracetamol did not alter fish behavior in response to acute exposure, nor induced alterations in hepatic glycogen metabolism, suggesting that there was no increase in energy demand, which consequently did not require an increase in plasma glycaemia (ISSEKURTZ, 2018). This effect may have been responsible for the lack of an increased demand for oxygen (ISSEKURTZ, 2018), thus not provoking the activation of anaerobic respiration. In this context, the LDH activity decreased because there was no increased metabolic demand to be supplied through anaerobiosis. However, in situations that require the use of anaerobic pathway for energy, such as hunting, predator escape, breeding, among others, this organism may be harmed (LI *et al*., 2009), given the extreme intensity of these stimuli that may favor oxidative stress and anaerobiosis. Inhibition of LDH activity may have a remarkable ecological significance, as it may lead to a reduction in muscle response to stimuli, which alters the behavior of organisms, leading to a difficulty in capturing prey, and making organisms exposed to such compounds more susceptible to being captured by predators (NUNES *et al*., 2015). Additionally, low levels of lactate dehydrogenase after exposure to paracetamol can prevent the organism from obtaining energy by anaerobic metabolism, which occurs in situations of hyperactivity involving hunting, predation and reproduction, which can leave the individual vulnerable and unable to obtain food and also to reproduce,

which may lead to a decline in the number of breeding individuals (SANTOS *et al*., 2018), with obvious population consequences.

It is important to address the fact that fish, especially during the acute exposure, were subjected to a short period (96h) of starvation, which is a requirement of the adopted testing guideline. However, and exactly to minimize the putative adverse effects of starvation on the physiological response of the animals, fish were fed the night prior to the onset of exposure, and were, until that moment, fed *ad libitum* during the quarantine period. In fact, starvation of fish may have metabolic consequences, but only after long periods. The study conducted by Carmen Hidalgo et al. (2017), found significant alterations in metabolism of the fish *Umbrina cirrosa*, but only a period of 4 weeks of starvation. Similarly, and according to Wang et al. (2019), the standard metabolic rate of fish of the species *Spinibarbus sinensis* starved for two weeks was not different from those that were fed continuously. In addition, and with a more phylogenetically similar fish species, the study conducted by Li *et al*. (2017) with the poecilid *G. affinis* showed that starvation only caused deleterious effects in metabolism of fish after periods of 60 days. For all mentioned studies, starvation was only capable of altering metabolic traits after periods that far exceeded the 4 days period here adopted. Consequently, the here-observed effects seem not to be related to starvation or to its consequences.

In addition, there was an increase in cholinesterase activity when fish were subjected to acute exposure to propranolol. According to the study by Alkondon *et al.* (1986), propranolol inhibits the activity of the cholinesterase enzyme in plasma and red blood cells of humans, as well as anticholinergic agents, such as atropine, act as antagonists of propranolol, reducing their hypotensive effect in rabbits. These findings indicate involvement and interaction between the cholinergic system and the pharmacodynamics of propranolol (ALKODON, 1986). However, this is not a general rule, and previous studies have shown that, for some species, there were no evidences that propranolol was able to cause anticholinesterase effects. Pereira *et al*. (2018) showed that acute and chronic exposures of *P. harpagos* to propranolol did not result in changes of cholinesterase activity of the central nervous system, i.e., it did not cause anticholinergic neurotoxic effects. According to the here obtained data, propranolol was responsible for a significant increase in cholinesterase activity in *P. harpagos*. The increase in cholinesterase activity obtained for *P. harpagos* exposed to propranolol was atypical, since the most frequent criterion adopted for the use of cholinesterases as effect criteria in ecotoxicology is their inhibition (ALKONDON *et al*., 1986), and not its induction.

The results obtained in the present study (especially for the chronic exposure) can also be related to a possible positive regulation of β-adrenergic receptors that are involved in a behavioral stress response to an aversive stimulus. This response was reported in rats when they were exposed to stressful conditions (PANDEY *et al*., 1994). Acute stress situations generated by several factors, including exposure to pollutants present in the water, generate a release of catecholamines (adrenaline and noradrenaline) by chromaffin cells in fish causing β-adrenergic receptors to move from the cytoplasm to the cell surface, increasing its sensitivity (SJOERD & BONGA, 1997). Catecholamines increase their concentration and exert their effects on β-adrenergic receptors acting on cell signal transduction pathways (AZEVEDO *et al*., 2009). β-blockers, such as propranolol, inhibit this effect (BORTOLLO & CONSOLIM-COLOMBO, 2009). However, it is possible that the low concentrations of propranolol that were used in our study probably were not able to block the receptors in response to this increased metabolism, in response to the chemical stress generated by drug exposure. For the chronic test, there were no changes in ChEs activity, which can be attributed to an adaptation of fish to the stressful condition. Changes in ChEs activity could generate growth inhibition, reproductive failure and death (SJOERD & BONGA, 1997), which did not occur in this study. It is thus possible to suggest that the here-adopted low and realistic concentrations of the drug were not enough to trigger any of the described mechanisms.

Chronic exposure to both drugs did not modify the here-tested enzymatic activities. Such short-term changes in enzymatic activities may be harmful to organisms, altering the preferential pathway of energy metabolism, and may induce behavioral changes that may compromise key features such as prey capture and predator escape. The results obtained for the enzyme lactate dehydrogenase after acute and chronic exposures to propranolol did not show any significant alteration, indicating that, under the adopted conditions, propranolol did not interfere in the anaerobic metabolism. The results of the present study did not point to a change in cholinesterase activity in fish exposed to paracetamol, suggesting that the selected concentrations or the exposure period were not sufficiently effective to generate neurotoxic nor inflammatory effects. On the other hand, according to the study performed by Pereira *et al.* (2018) with *P. harpagos*, acute exposure to paracetamol caused an increase in ChEs activity in the central nervous system. The authors suggested that an increase of the activity of serum cholinesterases after exposure to paracetamol might have occurred, because this compound can trigger inflammatory processes (NUNES, 2011; DAS, 2007). However, our data do not support this trend. In fact, AChEs activity was not altered when fish were exposed

to paracetamol after any of the exposure periods, leading to the conclusion that this exposure regimen did not trigger cholinergic neurotoxic responses. AChEs activity was only altered after acute exposure to propranolol, probably because of the low concentrations used in exposure to propranolol that were not enough to prevent metabolic augmentation response in response to stress generated exposure to the xenobiotic. Subsequently, for the chronic assay, the occurrence of an adaptive process is proposed, reestablishing the activity of this biomarker to its basal levels. Environmentally relevant levels of paracetamol caused alterations in lactate dehydrogenase activity, and this response was attributed to a preference of the organism to obtain energy through aerobic metabolism. Inhibition of LDH prevents energy production through anaerobic metabolism, which is an ecologically relevant result, since it adversely challenges the organism in situations of high activity such as hunting, predator flight, and reproduction, among others. Propranolol did not alter lactate dehydrogenase activity, at least at the concentrations and here-evaluated exposure periods.

Conclusions

Despite being theoretically more relevant, since they represent modes of exposures more realistic and closer to real environmental conditions, chronic exposures did not yield noteworthy alterations in all tested parameters. On the contrary, short term stress was evidenced by a series of physiological responses, which may be responsible for ulterior deleterious effects on exposed organisms, namely by modifying the preferential pathway of energy metabolism. Environmentally relevant levels of paracetamol caused alterations in lactate dehydrogenase activity, and this response was attributed to a preference of the organism to obtain energy through aerobic metabolism. Inhibition of LDH prevents energy production through anaerobic metabolism, which is an ecologically relevant result, since it adversely challenges the organism in situations of high activity such as hunting, predator flight, and reproduction, among others. Propranolol did not alter lactate dehydrogenase activity, at least at the concentrations and exposure periods here-evaluated. By acting this way, the here tested drugs (at least, paracetamol) may be ultimately responsible for behavioral changes, potentially implicated in altering key features of the normal activity of this species. Modifications in the normal behavior of *P. harpagos* are likely to end up being a causal factor in altered responses which may compromise prey capture and predator escape, with potential to have further consequences namely at the population level.

Acknowledgments

Bruno Nunes is hired by "ECO-R-pharmplast - Ecotoxicity of realistic combinations of pharmaceutical drugs and microplastics in marine ecosystems", Fundação para a Ciência e a Tecnologia, FCT (reference POCI-01-0145-FEDER-029203). Thanks are also due for the financial support to CESAM (UID/AMB/50017/2019), to FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020.

FIGURE LEGENDS

Figure 1. Data from exposure of *P. harpagos* to paracetamol and propranolol: acute effects on (a) (b) and chronic effects (c) (d) cholinesterase activity, determined both in dorsal muscle tissue, expressed in nmol minute^{-1} mg protein^{-1}. Values are the mean of 15 replicates, with the corresponding standard error bars. * - significant differences in relation to the control group, $p < 0.05$.

Figure 2. Data from exposure of *P. harpagos* to paracetamol and propranolol: acute effects (a) (b) and chronic effects (c) (d) on lactate dehydrogenase activity, determined both in dorsal muscle tissue, expressed in mmol minute^{-1} mg protein^{-1}. Values are the mean of 15 replicates, with the corresponding standard error bars. * - significant differences in relation to the control group, $p < 0.05$.

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Conflict of Interest

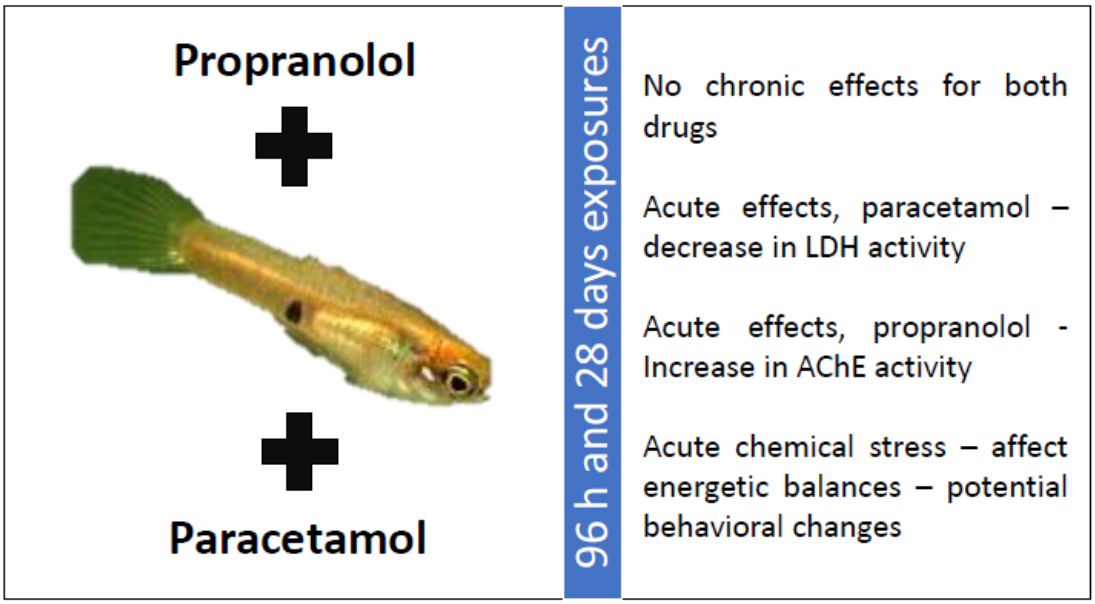
Dear Editor of Comparative Biochemistry and Physiology (part C),

Authors wish to confirm that there are no known conflicts of interest associated with this short communication ("Neurotoxic and respiratory effects of human use drugs on a Neotropical fish species, *Phalloceros harpagos*") and there has been no significant financial support for this work that could have influenced its outcome.

Sincerely yours

Bruno Nunes

Corresponding Author


Propranolol
Paracetamol
96 h and 28 days exposures
No chronic effects for both drugs
Acute effects, paracetamol – decrease in LDH activity
Acute effects, propranolol - Increase in AChE activity
Acute chemical stress – affect energetic balances – potential behavioral changes

HIGHLIGHTS

Acute exposure to paracetamol induced an enzymatic activity inhibition of lactate dehydrogenase in *Phaloceros harpagos*.

Acute exposure to propranolol triggered an increase in fish cholinesterase activity.

Chronic exposure to paracetamol or propranolol did not modify the enzymatic activities.

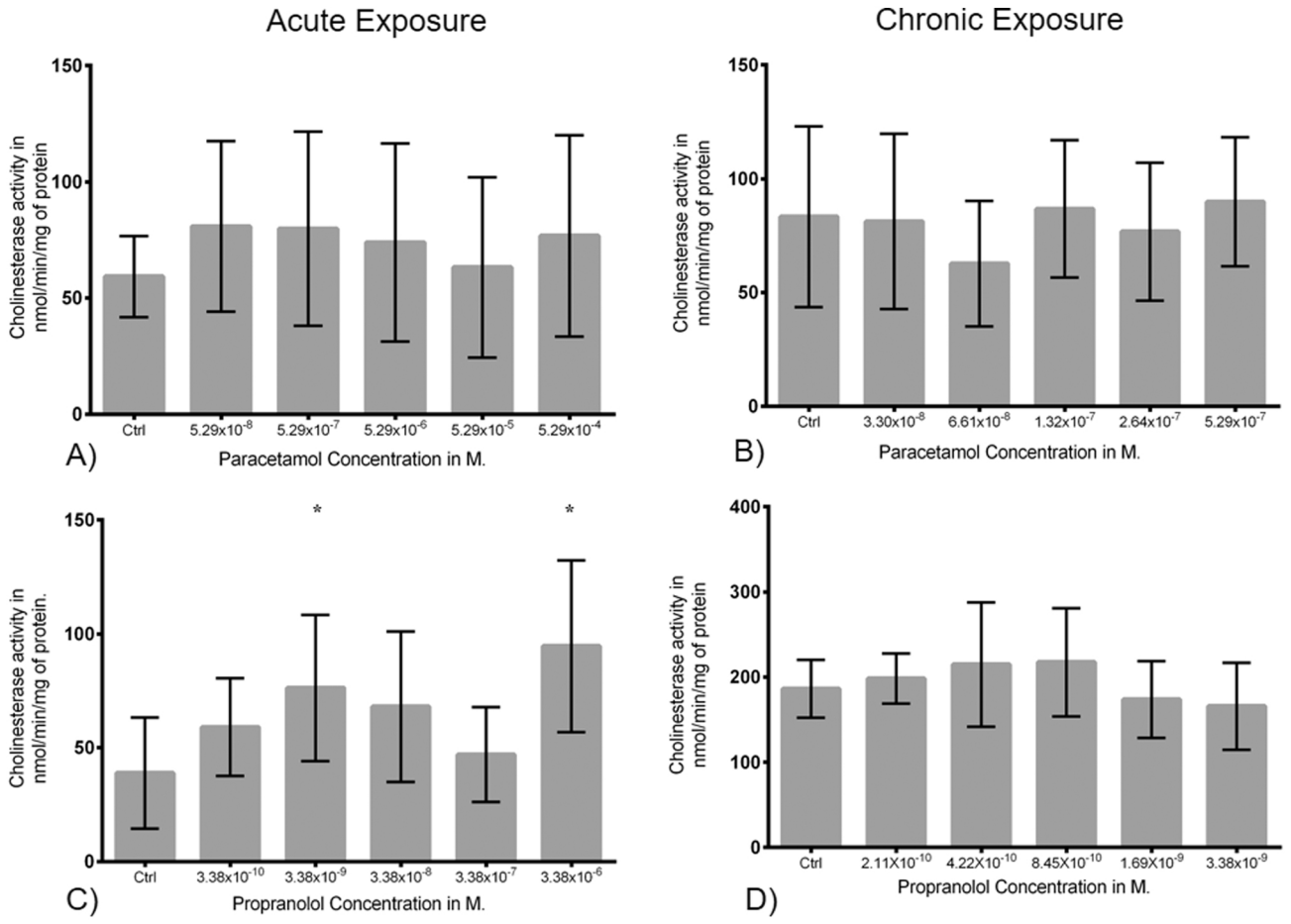

Acute Exposure
Chronic Exposure
Cholinesterase activity in nmol/min/mg of protein
150
100
50
0
Ctrl
5.29x10-8
5.29x10-7
5.29x10-6
5.29x10-5
5.29x10-4
A)
Paracetamol Concentration in M.
3.30x10-8
6.61x10-8
1.32x10-7
2.64x10-7
5.29x10-7
B)
Cholinesterase activity in nmol/min/mg of protein.
*
*
3.38x10-10
3.38x10-9
3.38x10-8
3.38x10-7
3.38x10-6
C)
Propranolol Concentration in M.
400
300
200
2.11X10-10
4.22X10-10
8.45X10-10
1.69X10-9
3.38x10-9
D)

Figure 1

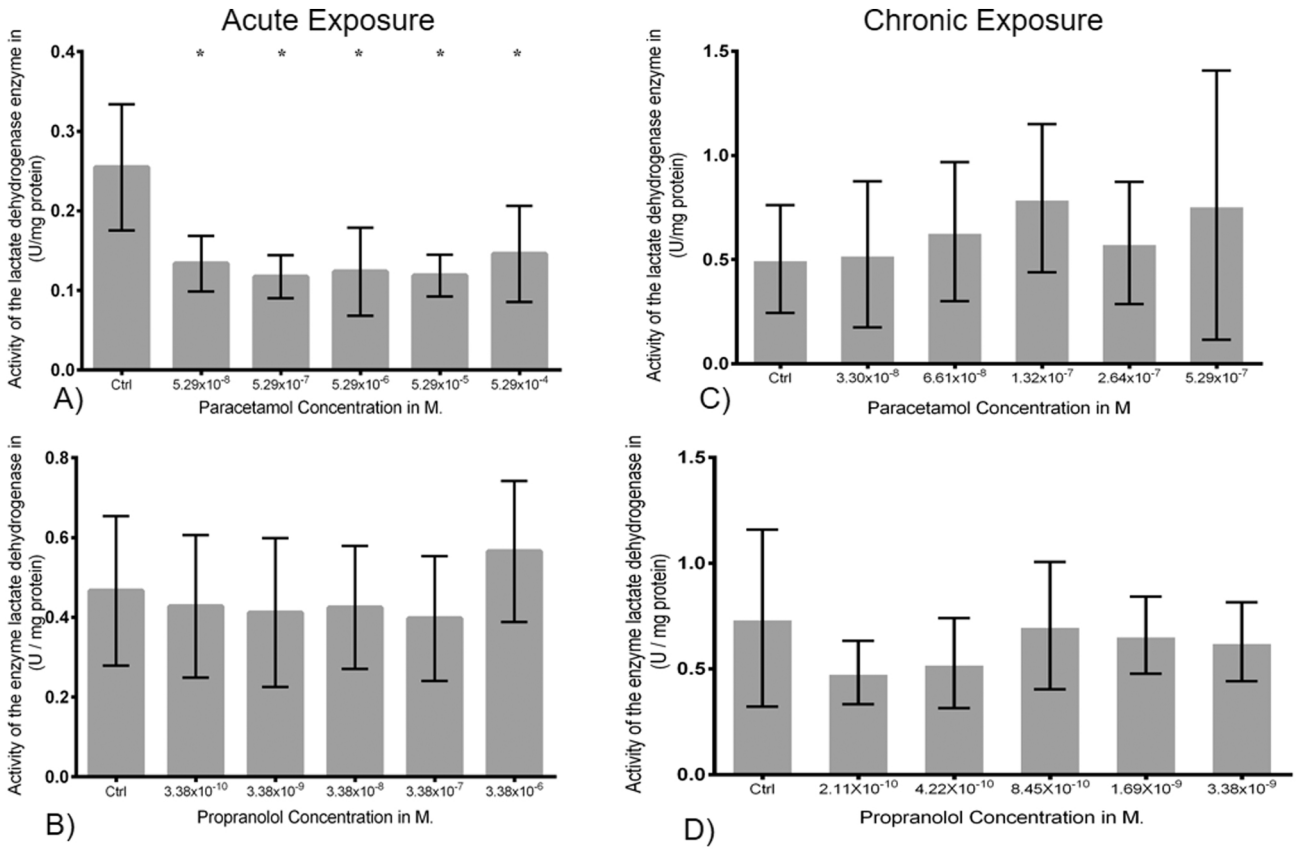

Acute Exposure
Activity of the lactate dehydrogenase enzyme in (U/mg protein)
0.4
0.3
0.2
0.1
0.0
*
*
*
*
*
Ctrl
5.29x10-8
5.29x10-7
5.29x10-6
5.29x10-5
5.29x10-4
A)
Paracetamol Concentration in M.
Chronic Exposure
Activity of the lactate dehydrogenase enzyme in (U/mg protein)
1.5
1.0
0.5
0.0
Ctrl
3.30x10-8
6.61x10-8
1.32x10-7
2.64x10-7
5.29x10-7
C)
Paracetamol Concentration in M
Activity of the enzyme lactate dehydrogenase in (U / mg protein)
0.8
0.6
0.4
0.2
0.0
Ctrl
3.38x10-10
3.38x10-9
3.38x10-8
3.38x10-7
3.38x10-6
B)
Propranolol Concentration in M.
Activity of the enzyme lactate dehydrogenase in (U / mg protein)
1.5
1.0
0.5
0.0
Ctrl
2.11X10-10
4.22X10-10
8.45X10-10
1.69X10-9
3.38x10-9
D)
Propranolol Concentration in M.

Figure 2