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Non-steroidal anti-inflammatory drugs and oxidative stress biomarkers in fish: a meta-analytic review

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ABSTRACT

Drug residues have been detected in aquatic environments around the world and non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most used classes. Therefore, it is important to verify the physiological effects of these products on exposed non-target organisms such as fish. Through a meta-analytic review, we evaluated the effects of NSAIDs on oxidative stress biomarkers in fish. Overall, Diclofenac was the most frequently tested drug in the systematically selected studies while acute and hydric exposure types were the most prevalent among these studies. The meta-analysis revealed that (1) chronic and subchronic exposures to NSAIDs decreased catalase (CAT) activity, and acute exposure increased glutathione peroxidase (GPx) activity; (2) hydric exposure increased GPx activity; (3) exposure to low concentrations of NSAIDs increased GPx and superoxide dismutase (SOD) activity; (4) Paracetamol exposure increased GPx and SOD activity and lipid peroxidation levels, but reduced glutathione S-transferase (GST) activity; (5) Diclofenac exposure increased GPx activity. In conclusion, our results demonstrated that fish are sensitive to NSAIDs exposure presenting significant alterations in oxidative stress biomarkers, especially in the GPx enzyme. This enzyme exhibits strong potential as a biomarker of NSAIDs exposure in fish. Paracetamol stood out as the NSAID that altered the largest number of oxidative stress biomarkers, drawing attention to its risk to fish. In contrast, ibuprofen did not change the biomarkers evaluated. These data demonstrate the important impact of emerging contaminants such as NSAIDs on aquatic organisms and the need for strategies to mitigate these effects.

1. Introduction

The rising demand for pharmaceutical products has significantly increased over the years, driven by advancements in the pharmaceutical industry and research [1,2]. However, the widespread use of these drugs has contributed to the increase in environmental contamination [2,3]. Several studies reported the presence of drug residues in sewage treatment plant effluents [4,5], groundwater [6,7], and even drinking water [8]. Among these pharmaceuticals, non-steroidal anti-inflammatory drugs (NSAIDs) [9] are particularly prevalent and frequently found in the environment. Common NSAIDs, such as Diclofenac, Paracetamol, and Ibuprofen, are widely used for treating pain, fever, and inflammation by inhibiting cyclooxygenase enzymes (COX-1 and COX-2) [2,10]. Because of their over-the-counter availability, the easy access to NSAIDs often leads to self-medication and excessive use [11]. Consequently, these drugs are continuously released into the aquatic environment through wastewater effluents and household wastes, among other input sources.

Once released into aquatic environments, NSAID exposure may have substantial adverse effects on non-target organisms at different levels of biological organization. For instance, studies conducted on fish have reported DNA damage [12], congenital malformation [13], alteration of locomotor activity [13], reduction in the number of leukocytes and thrombocytes, and oxidative stress [14]. Fish serve as important bioindicators of environmental pollution [15,16].

Oxidative stress arises due to an imbalance between reactive oxygen species (ROS) and the antioxidant system, which causes damage to vital macromolecules, including lipids, proteins, and DNA [17]. Key

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antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST), play essential roles in mitigating ROS-induced damage [17,18]. Briefly, SOD catalyzes the dismutation of the superoxide radical into hydrogen peroxide and oxygen [18]; GPx catalyzes the reduction of hydrogen peroxide and organic peroxides to their corresponding alcohols by converting GSH into glutathione disulfide [19]; CAT performs the catalysis of hydrogen peroxide into water and oxygen molecules [20]; and GST is a phase II detoxifying enzyme that catalyzes the conjugation of electrophilic substrates to GSH [21].

Given these concerns, meta-analysis evaluated the impact of NSAIDs on oxidative stress biomarkers in fish, focusing on key enzymes such as CAT, SOD, GPX, GST, and lipid peroxidation (LPO). Understanding these biochemical responses is crucial, as they serve as early indicators of physiological stress in non-target organisms, offering valuable insight into the broader ecological risks posed by NSAIDs. By identifying these early warning signals, this analysis seeks to deepen our understanding of the environmental impact of NSAIDs and contribute to the development of effective mitigation strategies.

2. Material and methods

This meta-analytic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [22]. Published articles were selected based on predefined inclusion and exclusion criteria. The included studies underwent a rigorous assessment of validity and quality, followed by a meta-analysis.

2.1. Search strategy

The search strategy was developed using the PECOS framework: P = Population, E = Exposure, C = comparison, O = outcomes, and S = study [23]. In this study, P = fish, E = NSAIDs (e.g., Diclofenac, Paracetamol, Ibuprofen, Naproxen, or Acetyl Salicylic Acid), C = no exposure to NSAIDs, O = alteration in oxidative stress biomarkers, and S = laboratory studies. This framework guided the research question "Does exposure to NSAIDs alter oxidative stress biomarkers in fish?"

Electronic databases including PubMed, Scopus, Embase, Science Direct, and Web of Science were searched for studies published up to October 2020. The following search strategies were applied across all databases:

Search 1: (fish AND environment)

Search 2: (oxidative stress OR antioxidant OR oxidant OR oxidative damage OR biomarker OR superoxide dismutase OR catalase OR glutathione OR glutathione peroxidase OR lipid peroxidation OR glutathione S-transferase)

Search 3: ("Non-steroidal anti-inflammatory drugs" OR NSAIDS OR Diclofenac OR Paracetamol OR Acetaminophen OR Ibuprofen OR Naproxen OR Acetylsalicylic acid).

2.2. Inclusion and exclusion criteria

To be included in this meta-analysis, all articles had to be peer-reviewed and meet the following criteria: (i) studies published in English; (ii) studies that address at least one of the following drugs: Diclofenac, Paracetamol, Acetaminophen, Ibuprofen, Naproxen, or Acetylsalicylic Acid (even if other drugs were also considered); (iii) *in vivo* studies using fish; (iv) studies that evaluated biochemical markers of oxidative stress (even if additional markers were also analyzed); (v) studies had to contain extractable data (*e.g.*, mean, standard deviation, sample size); (vi) studies that performed analyses exclusively in laboratory settings.

The exclusion criteria were: (i) *in vitro* studies; (ii) studies performed on animals other than fish; (iii) studies that focused on other drug classes other than NSAIDs; (iv) studies that evaluated only fish behavior; (v) studies that did not conduct analyses in a laboratory setting.

2.3. Study selection and data collection process

Following the database search, all retrieved articles were imported into Mendeley software, where duplicates were identified and removed. Subsequently, based on the title and abstract, articles that did not meet the inclusion criteria were excluded, and potentially eligible studies were selected for a more detailed evaluation.

Data were extracted independently by two authors (LHZJ and JFS). Discrepancies regarding the data extraction were resolved through discussion between the two reviewers, with a third reviewer (ICG) serving as a referee when necessary. Three categories of information were retrieved from the selected studies. First, citation details were recorded, including the title, authors, year of publication, and country. Second, study characteristics and experimental setup details were documented, such as, sample size, control group, fish species, NSAID tested, route and time of exposure, and analyzed tissue. Finally, quantitative data necessary for effect size calculations were extracted, including the mean or other central tendency measures, standard deviation, or standard error. Confidence intervals, standard error, or coefficients of variation were converted to standard deviations whenever feasible. When quantitative data were not reported in the text or tables, they were extracted from the figures using the free software Graph Grabber (version 2.0.2, Quintessa Software, 2017).

Most of the selected studies evaluated multiple exposure durations, NSAIDs concentrations, and biomarkers. Thus, data from each treatment within each study were extracted and coded to address the non-independence of estimates. Exposure durations were categorized as acute (up to 7 days), subchronic (up to 21 days), and chronic (over 28 days). NSAID concentrations were classified as low (100 μg/L or less) and high (greater than 100 μg/L)..

2.4. Meta-analysis

We used the natural logarithm of the response ratio (lnRR) to compare the means of the treatment and control groups. Positive lnRR values indicate increased levels and activities of the biomarkers reviewed, while negative lnRR values indicate decreased levels or inhibited activity. A separate model was fitted for each biomarker, incorporating factors (moderators) that might influence the biomarkers' responses to NSAID exposure.

Uni-moderator meta-regression models were performed for each of the following moderators: Time of exposure (three levels: acute, subchronic, and chronic); NSAID (three levels: Diclofenac, Paracetamol, and Ibuprofen), concentration (two levels: low and high), and exposure route (two levels: trophic and hydric). Linear mixed-effects meta-analytic models were used, with the lnRR effect size as the response variable and its relative variance (VSMD) as the sampling error. We also adjusted a variance-covariance matrix across all models to account for non-independence between effect sizes, which can arise when a single control group is compared to multiple treatments.

3. Results

3.1. Database and overall effects

The systematic search resulted in 143 records: 16 articles from PubMed, 19 from Science Direct, 54 from Scopus, 41 from the Web of Science, and 13 from Embase. All articles were transferred to Mendeley Desktop and after duplicates were removed (n = 68), 75 articles were selected for title and abstract screening. After reading titles and abstracts, 54 articles that did not meet the inclusion criteria were excluded, leaving 20 articles. Additionally, seven articles were included manually, resulting in a total of 27 articles (Fig. 1). Table 1S and Fig. 1S-5S (Supplementary Material) detail fish species, NSAIDS, exposure routes, concentrations, exposure times, tissues analyzed, biomarkers, and the main findings of the 27 selected papers.

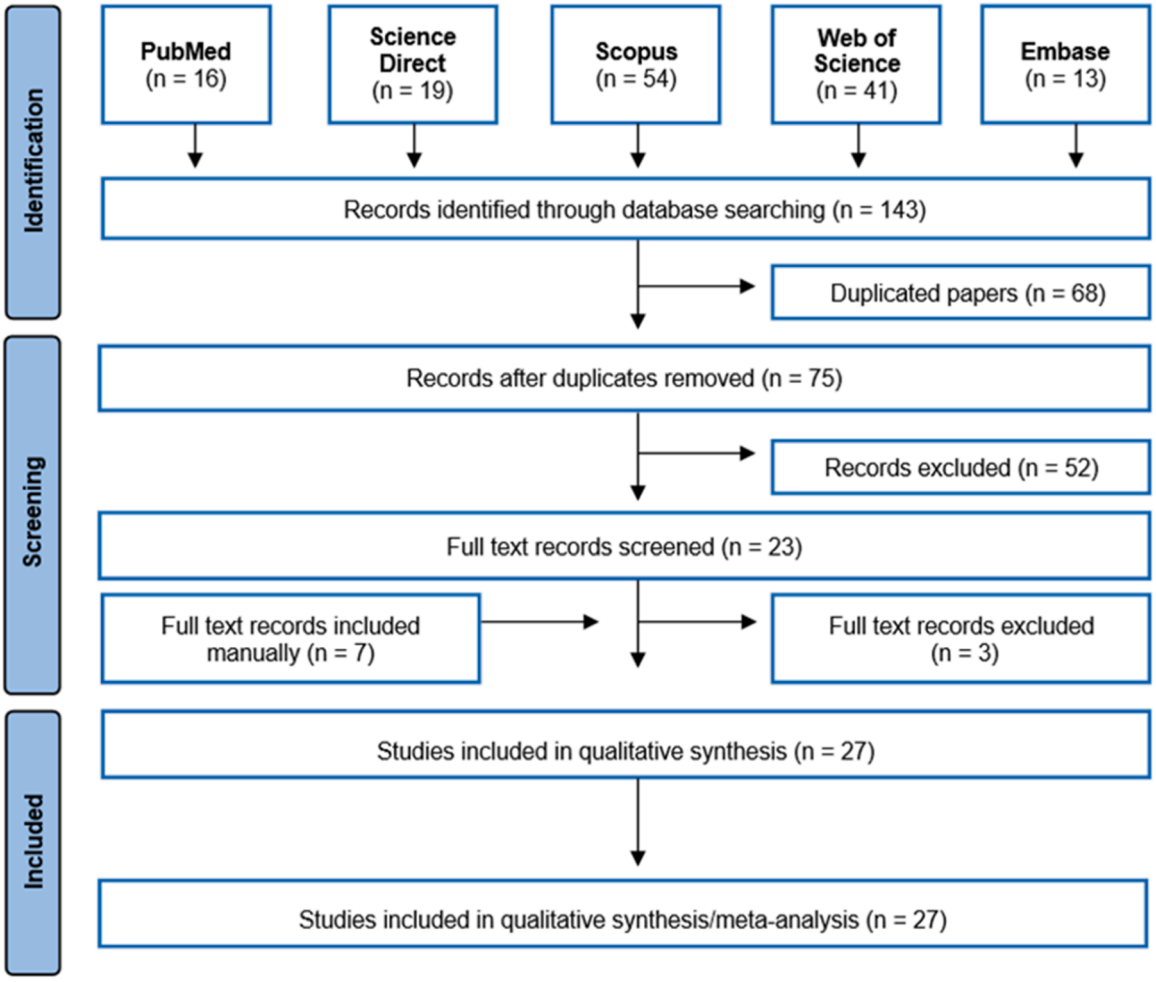

Fig. 1. Screening process summarized and formatted as a PRISMA flow diagram.

Overall, NSAIDs did not alter the biomarker responses, i.e., biomarker activity and levels from fish exposed to NSAIDs. Only GPx activity increased in fish exposed to NSAIDs (Figs. 2–7). Given the high

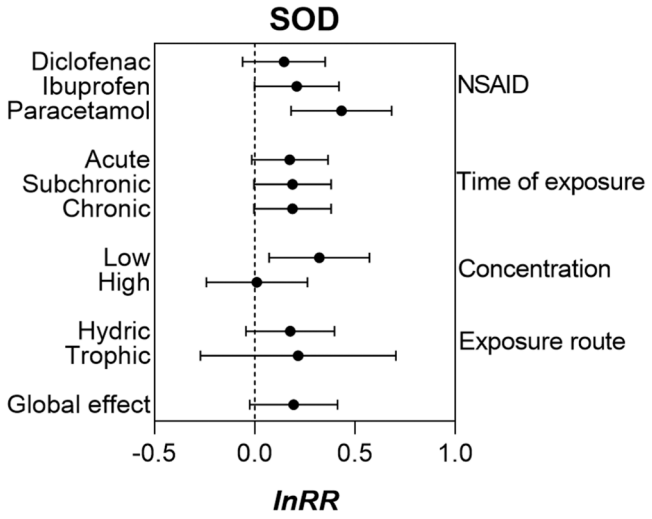

Fig. 2. Effects of NSAID exposure on fish SOD activity. For each experimental approach, the effects of specific NSAIDs, exposure duration, concentration, and exposure route are shown. Dots represent effect size estimates, and error bars indicate 95 % confidence intervals. Effects are considered significant if the 95 % confidence intervals do not cross the dashed line at 0.

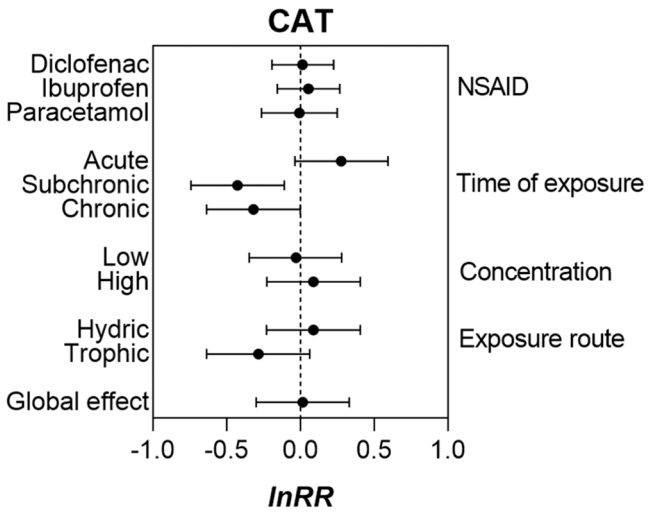

Fig. 3. Effects of NSAID exposure on fish CAT activity. For each experimental approach, the effects of specific NSAIDs, exposure duration, concentration, and exposure route are shown. Dots represent effect size estimates, and error bars indicate 95 % confidence intervals. Effects are considered significant if the 95 % confidence intervals do not cross the dashed line at 0.

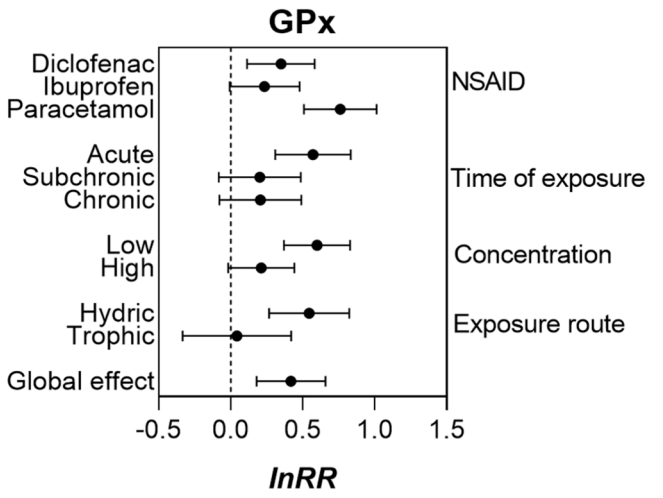

Fig. 4. Effects of NSAID exposure on fish GPx activity. For each experimental approach, the effects of specific NSAIDs, exposure duration, concentration, and exposure route are shown. Dots represent effect size estimates, and error bars indicate 95 % confidence intervals. Effects are considered significant if the 95 % confidence intervals do not cross the dashed line at 0.

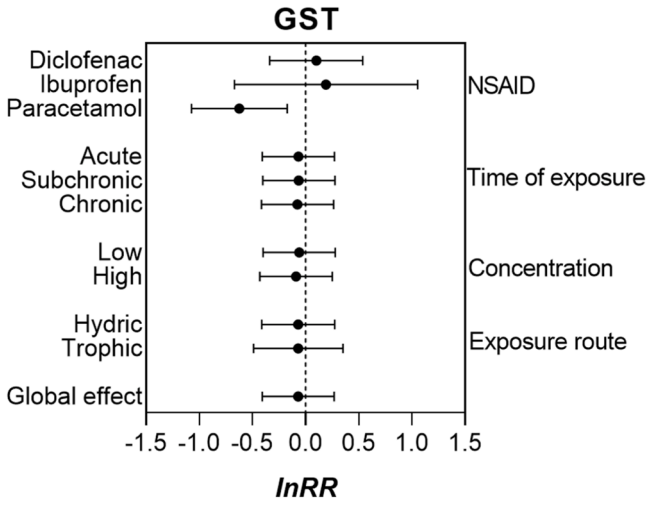

Fig. 5. Effects of NSAID exposure on fish GST activity. For each experimental approach, the effects of specific NSAIDs, exposure duration, concentration, and exposure route are shown. Dots represent effect size estimates, and error bars indicate 95 % confidence intervals. Effects are considered significant if the 95 % confidence intervals do not cross the dashed line at 0.

amount of heterogeneity, further analyses were conducted exploring differences among different NSAIDs, time of exposure, concentration, and route of exposure.

Tables 2S and 3S of the Supplementary Material provide additional meta-analysis parameters. Fig. 6S depicts funnel plots.

3.2. Specific NSAIDs effects on oxidative stress biomarkers

Exposure to Paracetamol increased fish SOD activity (Fig. 2; lnRR: 0.4329, CI: 0.1822 – 0.6837; $p = 0.0007$), GPx activity (Fig. 4; lnRR: 0.7612, CI: 0.5087 – 1.0137; $p < 0.00001$), and LPO levels (Fig. 7; lnRR: 0.5542, CI: 0.2822 – 0.8263; $p = 0.0001$). Conversely, Paracetamol exposure decreased GST activity (Fig. 5; lnRR: −0.6229, CI: −1.0734 – −0.1724; $p = 0.0067$) and tended to decrease GSH levels (Fig. 6; lnRR: −0.3677, CI: −0.7553 – 0.0199; $p = 0.0630$).

The exposure to Diclofenac increased GPx activity (Fig. 4; lnRR: 0.3493, CI: 0.1140 – 0.5846; $p = 0.0036$).

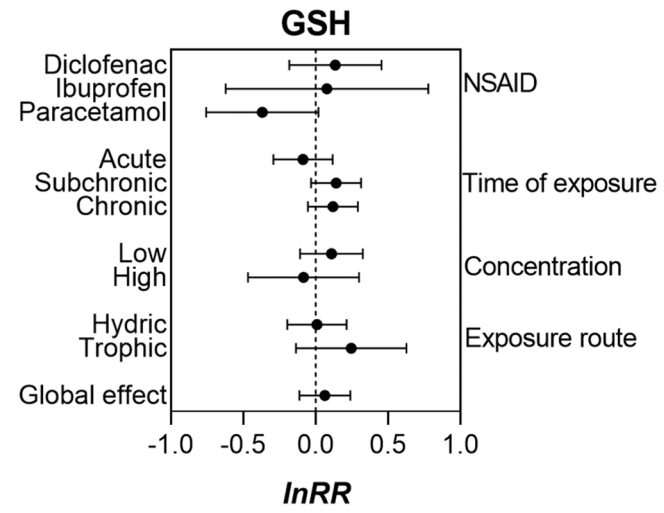

Fig. 6. Effects of NSAID exposure on fish GSH levels. For each experimental approach, the effects of specific NSAIDs, exposure duration, concentration, and exposure route are shown. Dots represent effect size estimates, and error bars indicate 95 % confidence intervals. Effects are considered significant if the 95 % confidence intervals do not cross the dashed line at 0.

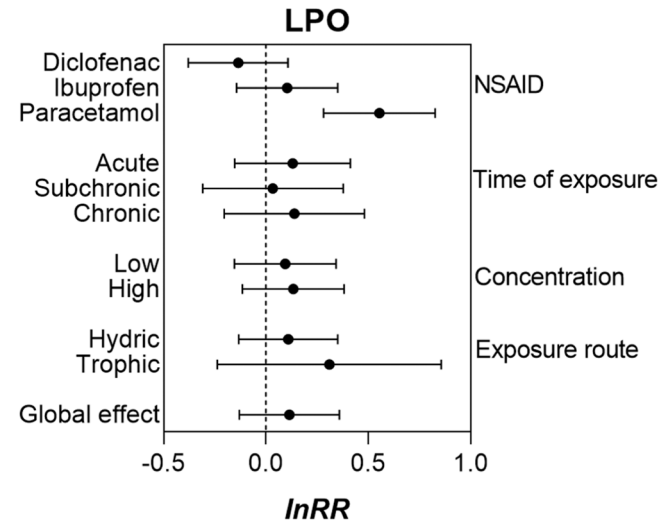

Fig. 7. Effects of NSAID exposure on fish GSH levels. For each experimental approach, the effects of specific NSAIDs, exposure duration, concentration, and exposure route are shown. Dots represent effect size estimates, and error bars indicate 95 % confidence intervals. Effects are considered significant if the 95 % confidence intervals do not cross the dashed line at 0.

Regarding Ibuprofen exposure, there was a tendency to increase SOD activity (Fig. 2; lnRR: 0.2100, CI: −0.0002 – 0.4201; $p = 0.0502$) and GPx activity (Fig. 4; lnRR: 0.2358, CI: −0.0077 – 0.4793; $p = 0.0577$).

The NSAIDs diclofenac, ibuprofen and paracetamol did not change CAT activity Fig. 3; $p > 0.05$).

3.3. Effects of exposure time to NSAIDs on oxidative stress biomarkers

Acute exposure (up to 7 days) increased GPx activity (Fig. 4; lnRR: 0.5723, CI: 0.3100 – 0.8346; $p < 0.00001$) and tended to increase SOD activity (Fig. 2; lnRR: 0.1754, CI: −0.0153 – 0.3662; $p = 0.0714$). Subchronic exposure (up to 21 days) decreased CAT activity (Fig. 3; lnRR: −0.4260, CI: −0.7431 – −0.1090; $p = 0.0085$) and tended to increase SOD activity (Fig. 2; lnRR: 0.1890, CI: −0.0034 – 0.3814; $p = 0.0542$). Chronic exposure (over 28 days) decreased CAT activity

(Fig. 3; lnRR: −0.3186, CI: −0.6356 – −0.0015; p = 0.0489) and tended to increase SOD activity (Fig. 2; lnRR: 0.1888, CI: −0.0036 – 0.3813; p = 0.0544).

3.4. Effects of concentration of NSAIDs on oxidative stress biomarkers

Exposure to low concentrations of NSAIDs (100 μg/L or less) increased SOD activity (Fig. 2; lnRR: 0.3220, CI: 0.0720 – 0.5720; p = 0.0116) and GPx activity (Fig. 4; lnRR: 0.5990, CI: 0.3692 – 0.8288; p < 0.00001). Interestingly, exposure to high concentrations of NSAIDs (greater than 100 μg/L) only tended to increase GPx activity (Fig. 4; lnRR: 0.2133, CI: −0.0167 – 0.4433; p = 0.0691).

3.5. Effects of exposure route to NSAIDs on oxidative stress biomarkers

Hydric exposure to NSAIDs increased GPx levels (Fig. 4; lnRR: 0.5451, CI: 0.2666–0.8288; p = 0.0001), while trophic exposure did not affect the oxidative stress biomarkers evaluated in this study.

4. Discussion

This study aimed to identify the most commonly studied emerging contaminants from the NSAIDs group in laboratory research and examine the oxidative stress biomarkers' response patterns in exposed fish. To achieve this, a meta-analysis was conducted on the effects of various NSAIDs (diclofenac, paracetamol, and ibuprofen) and several influencing factors, including exposure routes (hydric and trophic), exposure durations (acute, subchronic, and chronic), and concentration levels (low or high), on oxidative stress biomarkers such as SOD, CAT, GPx, GST, GSH, and LPO.

Our qualitative analysis (Table 1S) revealed that the biological effects of NSAIDs — namely diclofenac, ibuprofen, and paracetamol — were assessed using various oxidative stress biomarkers. To gain deeper insights into the effects of NSAIDs on fish, we conducted a meta-analysis. The findings indicate that NSAID exposure in non-target organisms like fish significantly alters the antioxidant system, especially SOD and GPx enzymes. However, substantial heterogeneity was observed across the results, which could be partially attributed to methodological and analytical variations among studies, as well as differences in the fish species and organs evaluated for each biomarker.

Regarding the route of exposure, most of the studies included in the meta-analysis assessed the effects of waterborne NSAIDs. This is an important moderator variable since the route of exposure can influence the toxicokinetic and toxicodynamic of a given xenobiotic [24]. In our analysis, fish exposed to NSAIDs via water showed an increase in GPx activity and a tendency (though not significant) for increased SOD activity. Notably, the activity of both enzymes was also increased in fish exposed to low concentrations (< 100 μg/L) of NSAIDs. This finding is concerning, as emerging contaminants like NSAIDs are more commonly detected in environmental concentrations below 100 μg/L [11,25]. A recent narrative review observed that NSAIDs are commonly found in aquatic environments at concentrations below 2 μg/L. At these concentrations, fish already exhibit damage in osmoregulation, markers of oxidative stress, and immune functions [26]. This demonstrates that even at low concentrations, commonly found in the environment, NSAIDs can affect biomarkers in aquatic organisms and underscores the need to develop new techniques to remove these emerging contaminants from the environment.

Exposure duration indicated that CAT activity decreased in fish exposed to NSAIDs subchronically and chronically but increased with acute exposure. This variation may represent an adaptive response to acute exposure, while subchronic and chronic exposure could suggest a direct interaction between NSAIDs and the enzyme or inhibition due to an excess of the substrate (i.e., hydrogen peroxide). This is plausible given that CAT exhibits unusual kinetic properties and does not follow the standard Michaelis–Menten model [27,28]. However, further studies are needed to confirm these hypotheses, including docking molecular simulations and evaluations of the enzyme's kinetic properties in fish.

In contrast, GPx and SOD activity increased, or showed a tendency to increase, in fish exposed to NSAIDs, regardless of exposure duration. This rise in enzyme activities seems to be an adaptive response aimed at mitigating the reactive oxygen species (ROS) generated by NSAIDs [29-31]. Supporting our hypothesis, the meta-analysis found no significant effect of NSAID exposure time on LPO levels.

Meta-regressions assessing the influence of individual NSAIDs indicated that Paracetamol exposure caused the most pronounced effects. Fish exposed to Paracetamol showed an increase in GPx and SOD activities and LPO levels while GST activity and GSH levels decreased (or tended to decrease). The mechanisms of Paracetamol overdose toxicity, particularly regarding hepatotoxicity, are well understood in mammals [32,33]. However, it seems that non-target organisms, such as fish, may be more sensitive to Paracetamol exposure, as alterations in oxidative stress biomarkers were noted even at low concentrations [34,14,35]. Interestingly, an increase in GPx and SOD activities was also observed in fish exposed to Ibuprofen and Diclofenac. While paracetamol showed pronounced effects on the analyzed oxidative stress biomarkers, diclofenac was the most commonly evaluated drug in studies (57.8 %). This is likely due to concerns about its impact on non-target organisms. In 2015, diclofenac was added to the European Union's list of priority substances, a framework designed to monitor the environmental concentrations of harmful compounds across Europe [36].

5. Conclusion

Our results demonstrate that GPx appears to be the most responsive biomarker and could serve as an indicator of NSAIDs exposure in laboratory studies. Paracetamol was the NSAID that altered the largest number of oxidative stress biomarkers, drawing attention to its risk to fish. In contrast, ibuprofen did not change the biomarkers evaluated. Further research is needed to explore the effects of other NSAIDs on fish. Notably, in our systematic search, we did not identify studies investigating the effects of other NSAIDs such as Naproxen and/or Acetylsalicylic acid in fish.

Furthermore, it was possible to verify that NSAIDs can cause oxidative stress in several fish species, altering homeostasis. These effects can have important ecological impacts. These results can provide support and direction for future ecotoxicological assessments of fish contamination by drugs from the NSAID group, allowing for more direct and effective detection of effects.

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CRediT authorship contribution statement

Juliana Ferreira Silva: Writing – original draft, Investigation, Data curation. **Manuela Santos Santana:** Writing – review & editing, Formal analysis. **Luiz Henrique Zaniolo Justi:** Writing – original draft, Investigation, Data curation, Conceptualization. **Izonete Cristina Guiloski:** Writing – review & editing, Supervision, Conceptualization. **Meire Ellen Pereira:** Data curation. **Cláudia Sirlene Oliveira:** Writing – review & editing. **Henrique Aparecido Laureano:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2025.101910.

Data availability

Data will be made available on request.

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