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OXIDATIVE AND ANTIOXIDANT RESPONSES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) CARDIAC TISSUE FOLLOWING CHLORAMINE-T EXPOSURE

Halina TKACZENKO, Doctor of Biological Sciences
https://orcid.org/0000-0003-3951-9005
Natalia KURHALUK, Doctor of Biological Sciences
https://orcid.org/0000-0002-4669-1092
Institute of Biology, Pomeranian University in Słupsk, Słupsk, Poland
Joanna GRUDNIEWSKA, Ph.D.,
https://orcid.org/0000-0002-4272-8337
Department of Salmonid Research, National Inland Fisheries Research Institute, Rutki, Poland

Chloramine-T is a widely used disinfectant in aquaculture for controlling bacterial and parasitic infections. While its antimicrobial efficacy is well established, the sublethal effects of Chloramine-T on fish cardiac tissue are not well understood. This study investigated the impact of short-term Chloramine-T baths on oxidative stress biomarkers and antioxidant defence mechanisms in the hearts of rainbow trout (Oncorhynchus mykiss). The fish were exposed to 9 mg/L chloramine-T in three 20-minute baths, administered at three-day intervals. Cardiac tissue was then analysed for lipid peroxidation (TBARS), protein oxidation (aldehydic and ketonic derivatives of oxidatively modified proteins, OMPs), total antioxidant capacity (TAC) and enzymatic antioxidant activities (SOD, CAT, GR and GPx). Exposure to chloramine-T significantly decreased aldehydic (56.1%) and ketonic (53.9%) protein oxidation products ($p < 0.001$), while there was a non-significant reduction in lipid peroxidation. Enzymatic antioxidant activities remained largely unchanged, with a mild non-significant increase in SOD and a modest rise in TAC. Correlation and regression analyses revealed a strong correlation between TBARS and OMPs ($r = 0.99$, $p < 0.001$) and identified glutathione reductase as the main predictor of lipid peroxidation ($\beta = 0.36$, $p = 0.04$). These results suggest that, at the tested concentration, chloramine-T does not induce harmful oxidative stress in trout hearts, but rather triggers adaptive redox modulation, particularly via glutathione-dependent pathways. These findings provide valuable insights into cardiac redox homeostasis in fish exposed to disinfectants and support the careful therapeutic use of chloramine-T in aquaculture.

Keywords: Chloramine-T; rainbow trout; oxidative stress; cardiac tissue; antioxidant enzymes; glutathione reductase; protein oxidation; lipid peroxidation

ОКСИДАТИВНА ТА АНТИОКСИДАНТНА ВІДПОВІДЬ У СЕРЦЕВІЙ ТКАНИНІ РАЙДУЖНОЇ ФОРЕЛІ (*ONCORHYNCHUS MYKISS* WALBAUM) ПІСЛЯ ЕКСПОЗИЦІЇ НА ХЛОРАМІН-Т

Галина ТКАЧЕНКО, доктор біологічних наук, професор
https://orcid.org/0000-0003-3951-9005
Наталія КУРГАЛЮК, доктор біологічних наук, професор
https://orcid.org/0000-0002-4669-1092
Інститут біології, Поморський університет у Слупську, Польща
Йоанна ГРУДНЄВСЬКА, кандидат наук,
https://orcid.org/0000-0002-4272-8337
Інститут рибальства імені Станіслава Саковича, Руткі, Жуково, Польща

Хлорамін-Т є широко використовуваним дезінфекційним засобом в аквакультурі для контролю бактеріальних та паразитарних інфекцій. Хоча його антимікробна ефективність добре задокументована, сублетальні ефекти хлораміну-Т на серцеву тканину риб залишаються недостатньо вивченими. У цьому дослідженні оцінювали вплив короткочасних ванн з хлораміном-Т на біомаркери оксидативного стресу та механізми антиоксидантного захисту у серці райдужної форелі (Oncorhynchus mykiss). Риб піддавали впливу хлораміну-Т у концентрації 9 мг/л у трьох 20-хвилинних ваннах з інтервалом у три дні. Серцеву тканину аналізували на рівень перекисного окиснення ліпідів (TBARS), окиснення білків (альдегідні та кетонові похідні окиснено модифікованих білків, OMP), загальну антиоксидантну здатність (TAC) та активність антиоксидантних ферментів (SOD, CAT, GR і GPx). Експозиція на хлорамін-Т призвела до значного зниження рівня альдегідних (на 56,1%) та кетонових (на 53,9%) продуктів окиснення білків ($p < 0,001$), тоді як зниження рівня перекисного окиснення ліпідів було статистично незначущим. Активність антиоксидантних ферментів залишалася переважно незмінною, з легким незначущим підвищенням активності SOD та помірним зростанням TAC. Кореляційний та регресійний аналізи виявили сильну кореляцію між TBARS і OMP ($r = 0,99$, $p < 0,001$) та визначили глутатіонредуктазу як основний предиктор перекисного окиснення ліпідів ($\beta = 0,36$, $p = 0,04$). Отримані результати свідчать про те, що при дослідженій концентрації хлорамін-Т не викликає шкідливого оксидативного стресу в серці форелі, а навпаки – активує адаптивну редокс-модуляцію, зокрема через глутатіонзалежні шляхи. Ці дані надають цінну інформацію про редокс-гомеостаз серцевої тканини риб, підданих дії дезінфекційних засобів, і підтримують обережне терапевтичне використання хлораміну-Т в аквакультурі.

Ключові слова: хлорамін-Т; райдужна форель; оксидативний стрес; серцева тканина; антиоксидантні ферменти; глутатіонредуктаза; окиснення білків; перекисне окиснення ліпідів.

Introduction. Disinfectants are routinely used in aquaculture to control bacterial, fungal and parasitic infections that can threaten the health of fish and reduce production efficiency (Abd El-Hack M. E. et al., 2022). Chloramine-T (sodium p-toluenesulfonchloramide) is one of the most widely used disinfectants due to its high efficacy, broad antimicrobial spectrum and relatively low toxicity when used correctly (Nayak Y. N. et al., 2022). It is particularly effective in treating gill diseases and external infections caused by *Flavobacterium* and *Ichthyobodo* species (Powell, M. D. et al., 1994; Ostland, V. E. et al., 1995; Altinok I., 2004). However, despite its recognised benefits,

chloramine-T is a strong oxidising compound capable of releasing active chlorine species, such as hypochlorous acid. These can react with cellular macromolecules and potentially disturb redox homeostasis in exposed fish tissues (Powell M. D. and Perry, S. F., 1999; Kuklina I. et al., 2014). This oxidative potential raises concerns about its sublethal effects on non-target organs, particularly in scenarios involving repeated or prolonged exposure, which are common in intensive aquaculture systems (Kumar P. et al., 2015; Tkachenko H. et al., 2021).

Fish exposed to oxidative agents, including disinfectants, experience an imbalance between the production of reactive oxygen species (ROS) and the effectiveness of antioxidant defence systems. ROS, including superoxide anion ($O_2^{-}\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH), can damage membrane lipids, proteins, and nucleic acids, causing structural and functional cellular impairment (Carvan M.J. and Di Giulio R.T., 2015; Hoseinifar S. H. et al., 2021; Juan C. A., 2021). Fish possess a complex antioxidant system to counteract this, consisting of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), as well as non-enzymatic components including glutathione (GSH), ascorbate and carotenoids (Srikanth K. et al., 2013; Atli G. et al., 2016). These defence mechanisms work together to detoxify ROS and repair oxidative damage, thereby maintaining tissue homeostasis. Disruption to this balance is indicated by elevated levels of biomarkers of oxidative damage, including 2-thiobarbituric acid reactive substances (TBARS) and oxidatively modified proteins (OMPs). Recent studies have also highlighted lactate dehydrogenase (LDH) activity as a sensitive indicator of tissue-specific oxidative damage, particularly in metabolically active organs such as the heart (Tkachenko H. et al., 2021).

Although chloramine-T is widely used in aquaculture, research examining its sublethal biochemical and physiological effects is limited, especially with regard to its impact on cardiac tissue, which is highly susceptible to redox imbalance. The fish heart is a metabolically active organ with high oxygen consumption, making it susceptible to oxidative stress caused by environmental and pharmacological factors (Leef M. J. et al., 2007; Tkachenko H. et al., 2013). Previous research has primarily focused on the responses of the gills, liver and kidneys to exposure to chloramine-T, while the cardiac effects, especially those involving oxidative biomarkers, are poorly understood. A recent study by Tkachenko H. et al. (2021) revealed substantial changes in LDH activity in the cardiac tissue of salmonids after chloramine-T exposure, indicating potential metabolic disturbances at sublethal concentrations. Evaluating the heart's response is essential for understanding not only systemic redox regulation, but also the safety margin of disinfection treatments under aquaculture conditions. Such assessments are crucial for developing evidence-based guidelines that balance antimicrobial efficacy with the physiological welfare of cultured fish.

Given the increasing use of chloramine-T in fish farming, it is important to establish whether exposure to it compromises the physiology of fish or triggers the activation of adaptive antioxidant mechanisms at therapeutic doses. This study investigated the effects of chloramine-T baths on biomarkers of oxidative stress and parameters of the antioxidant defence system in the cardiac tissue of rainbow trout (*Oncorhynchus mykiss*), a species widely used in aquaculture and ecotoxicological research. Specifically, we aimed to: (i) quantify changes in lipid and protein oxidation biomarkers (TBARS and OMP); (ii) assess alterations in the activities of major antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx)), as well as total antioxidant capacity (TAC); and (iii) analyse the relationships among these parameters using correlation,

regression, and effect size analyses. The obtained data offer valuable insights into the redox balance mechanisms of fish cardiac tissue when exposed to an oxidant commonly used in aquaculture disinfection practices. The data may also contribute to the refinement of guidelines for the safe and effective use of chloramine-T in fish health management.

Materials and methods.

Fish. Twenty clinically healthy rainbow trout (*Oncorhynchus mykiss* Walbaum) were used in the experiments. The study was carried out at the Department of Salmonid Research at the Inland Fisheries Institute in Olsztyn, Poland. The experiments were performed at a water temperature of 16 ± 2 °C and a pH of 7.5. The dissolved oxygen level was approximately 12 ppm, with an additional oxygen supply ensuring optimal environmental conditions. The fish were fed a commercial trout diet at a rate of 1.5% of their body weight once daily and acclimated to laboratory conditions for 14 days prior to the start of the experiment. All biochemical assays were conducted at the Department of Zoology and the Department of Animal Physiology at the Institute of Biology, Pomeranian University in Słupsk, Poland.

Experimental design. The fish were divided into two groups of ten fish each to maintain equal biomass, and held in 250-litre square tanks supplied with the same water as during the acclimation period. On alternate days, the water supply to each tank was stopped to allow controlled exposure. In the disinfectant exposure group, rainbow trout (n = 10) were exposed to chloramine-T at a final concentration of 9 mg L^{-1}. The control group (n = 10) was handled in the same way, but without the addition of chloramine-T. The fish were bathed for 20 minutes, and this procedure was repeated three times at three-day intervals. Two days after the final bath, the fish were sampled for biochemical analysis. All procedures were conducted in accordance with the Local Ethical Committee for Animal Experiments' guidelines (Inland Fisheries Institute, Olsztyn, Poland), ensuring minimal stress and humane treatment of the fish throughout the experiment. Fish were not anaesthetised before tissue sampling to avoid potential alterations to the analysed parameters.

Tissue collection and sample preparation. Two days after the final bath, the rainbow trout were euthanised humanely by decapitation. Immediately afterwards, the heart was excised in situ under aseptic conditions. The organs were then perfused with an ice-cold isolation buffer to remove any remaining blood. This buffer solution consisted of 100 mM Tris-HCl at pH 7.2. All reagents used were of analytical grade and obtained from Sigma-Aldrich (St. Louis, Missouri, USA) and Avantor Performance Materials Poland S.A. (Gliwice, Poland).

The cardiac tissue was then homogenised using a motor-driven pestle in a glass H500 homogeniser, and the samples were kept in an ice–water bath to prevent protein degradation. This resulted in a 1:9 (weight/volume) homogenate. The homogenates were then centrifuged at 3,000 rpm (approximately 1,000×g) for 15 minutes at 4 °C. After centrifugation, the supernatant was collected and stored at −25 °C until biochemical analysis. Before analysis, all samples were thawed only once to minimise protein denaturation and artefacts of oxidation.

The protein content of the supernatant was determined using the Bradford method (Bradford, M. M., 1976), with bovine serum albumin (BSA) used as the standard. Absorbance was measured at 595 nm using a UV–Vis spectrophotometer (Spekol 11, Carl Zeiss, Jena, Germany). All assays were performed in duplicate at 22 ± 0.5 °C and biochemical reactions were initiated by adding the tissue supernatant to the respective reaction mixtures. Enzyme activity and oxidative stress biomarkers were expressed per milligram of protein to enable comparison between samples.

Specific assay conditions for each parameter are described in the following subsections.

2-Thiobarbituric acid reactive substances (TBARS) assay. Lipid peroxidation levels were assessed by measuring the concentration of 2-thiobarbituric acid reactive substances (TBARS) according to the method of Buege J.A. and Aust S.D. (1978), with minor modifications. This assay is based on the reaction between 2-thiobarbituric acid (TBA; Sigma-Aldrich, St. Louis, MO, USA) and malondialdehyde (MDA) or similar aldehydic products of lipid peroxidation. Under high temperature and acidic conditions, TBA reacts with MDA to form a pink chromogen with a maximal absorbance at 532 nm.

Briefly, 0.5 ml of supernatant was mixed with 2.5 ml of TBA reagent (0.375% TBA, 15% trichloroacetic acid, and 0.25 N HCl) and heated in a boiling water bath for 15 min. After cooling on ice, samples were centrifuged at 3,000 × g for 10 min to remove precipitated proteins. The absorbance of the supernatant was measured at 532 nm against reagent blanks using a UV-Vis spectrophotometer (Spekol 11, Carl Zeiss, Jena, Germany).

The TBARS concentration was calculated using the molar extinction coefficient for the MDA-TBA complex ($\varepsilon = 1.56 \times 10^5$ $M^{-1} \cdot cm^{-1}$) and expressed as nanomoles of MDA equivalents per milligram of protein.

Assay for carbonyl groups in oxidatively modified proteins. Carbonyl groups were determined as a marker of protein oxidation using the method of Levine R. L. et al. (1990) with some modifications. Briefly, homogenate supernatant aliquots were incubated with 10 mM 2,4-dinitrophenylhydrazine (DNPH; Sigma-Aldrich, St. Louis, Missouri, USA) in 2 M hydrochloric acid (HCl) for one hour at room temperature and in the dark. Blank samples were prepared under identical conditions, but without DNPH.

The proteins were then precipitated using 20% trichloroacetic acid (TCA), after which the mixture was centrifuged at 3,000 g for 20 minutes at 4 °C. The protein pellet was washed three times with a mixture of ethanol and ethyl acetate (1:1, v/v) to remove any remaining reagents or lipids. The pellet was then incubated at 37 °C in an 8 M urea solution until completely resuspended. The carbonyl content was measured spectrophotometrically at 370 nm for aldehydic derivatives (OMP_{370}) and at 430 nm for ketonic derivatives (OMP_{430}), using a molar extinction coefficient of 22,000 $M^{-1} \cdot cm^{-1}$. The results were expressed as nmol of carbonyl groups per mg of protein (Levine et al., 1990).

Total antioxidant capacity (TAC) assay. TAC was determined spectrophotometrically using the Tween 80 oxidation method (Galaktionova L. P. et al., 1998). Absorbance was measured at 532 nm and TAC values were expressed as a percentage relative to the control oxidation value.

Assays of antioxidant enzyme activity. Superoxide dismutase (SOD; EC 1.15.1.1) activity in the sample supernatant was determined spectrophotometrically according to the method of Kostiuk V. A. et al. (1990). The principle of this method is based on SOD inhibiting the autooxidation of quercetin in the reaction system, and the enzyme activity was expressed in units per milligram of protein.

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the rate of hydrogen peroxide decomposition at 410 nm, as described by Koroliuk M. A. et al. (1988). The reaction mixture contained a phosphate buffer solution (50 mM, pH 7.2) and a freshly prepared 0.03% H_2O_2 solution. One unit of CAT activity was defined as the amount of enzyme decomposing 1 μmol of H_2O_2 per minute at 25 °C, and the enzyme activity was expressed in units per milligram of protein.

Glutathione reductase (GR; EC 1.6.4.2) activity was assayed as described by Glatzle D. et al. (1974), with minor modifications. This method is based on the reduction

of oxidised glutathione (GSSG) to reduced glutathione (GSH) in the presence of $NADPH_2$. The rate of NADPH oxidation was monitored via a decrease in absorbance at 340 nm, and enzyme activity was expressed as μmol of $NADPH_2$ oxidised per minute per milligram of protein.

The activity of glutathione peroxidase (GPx; E.C. 1.11.1.9) was determined by detecting the non-enzymatic utilisation of reduced glutathione (GSH) as the reacting substrate after incubation with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), according to the method described by Moin V. M. (1986). Absorbance was recorded at 412 nm. The reaction was carried out in a phosphate buffer solution (pH 7.2, 50 mM) containing 1 mM EDTA and 1 mM sodium azide to inhibit catalase activity. GPx activity was expressed as μmol of GSH oxidised per minute per milligram of protein.

All spectrophotometric measurements were performed at 22 ± 0.5°C using a UV–Vis spectrophotometer (Spekol 11, Carl Zeiss, Jena, Germany). Enzyme activities were normalised to the total protein concentration, which was determined using the Bradford method (Bradford M. M., 1976).

Statistical analysis. All data are presented as means ± standard deviation (SD). Prior to statistical analysis, the data were tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. When the assumptions of parametric analysis were met, comparisons between the control group and the group exposed to chloramine T were performed using a Student's t-test for independent samples. In cases where the data distribution deviated from normality, the non-parametric Mann-Whitney U test was applied (Stanisz A., 2006, 2007).

The level of statistical significance was set at $p < 0.05$. Effect sizes for group differences were calculated using Cohen's d to evaluate the magnitude of treatment effects beyond statistical significance. The interpretation of Cohen's d values followed conventional thresholds: 0.2 (small), 0.5 (medium) and ≥0.8 (large).

Correlations between oxidative stress biomarkers and antioxidant parameters were evaluated using the Pearson correlation coefficient (r), and the strength of the association was classified as follows: weak ($r < 0.3$), moderate ($0.3 \leq r < 0.7$), or strong ($r \geq 0.7$). When the data did not meet the assumptions of linearity or homoscedasticity, Spearman's rank correlation was used as a non-parametric alternative.

Multiple linear regression analysis was performed to identify the most important enzymatic predictors of lipid peroxidation (TBARS levels). The regression model included SOD, CAT, GR, GPx and TAC as independent variables, with TBARS as the dependent variable. Multicollinearity was checked using the variance inflation factor (VIF; VIF <5 was considered acceptable). The model fit was evaluated using the coefficient of determination (R^2), adjusted R^2, and the statistical significance of standardised beta coefficients (β).

All statistical analyses were carried out using Statistica 13.3 (TIBCO Software Inc., Palo Alto, USA). Graphs were generated in Microsoft Excel, and significance levels are indicated in the figure and table.

Results. Figure 1 shows the levels of TBARS (a key indicator of lipid peroxidation), as well as the levels of aldehydic and ketonic derivatives of oxidatively modified proteins (OMPs) and total antioxidant capacity (TAC), in the cardiac tissue of rainbow trout that were bathed in chloramine-T.

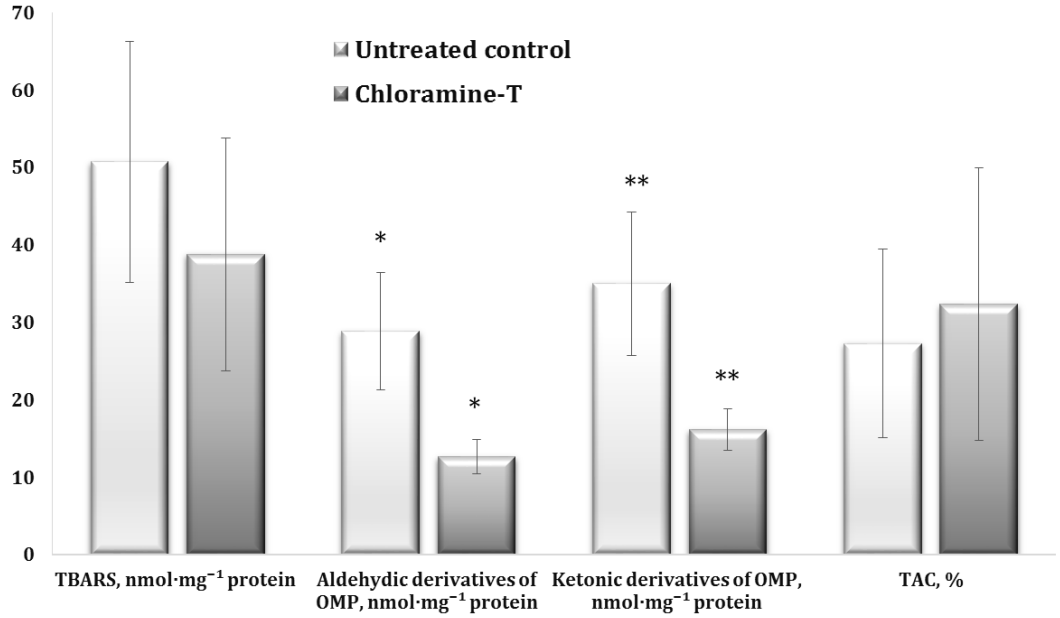

Fig. 1. **Levels of TBARS (a key indicator of lipid peroxidation), aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the cardiac tissue of rainbow trout bathed in chloramine-T.**

Data are presented as means ± S.D. (n = 10).
**and ** Significant differences (p < 0.05) between the untreated control group and the group bathed in chloramine-T.*

Exposure to chloramine-T baths resulted in significant changes to oxidative stress biomarkers and antioxidant parameters in the hearts of rainbow trout (Fig. 1). The mean TBARS level in the control group was 50.76 ± 15.55 nmol MDA·mg^{-1} protein. In the chloramine-T group, this decreased by −23.6% to 38.80 ± 15.06 nmol MDA·mg^{-1} protein ($p = 0.098$), indicating a non-significant reduction in lipid peroxidation. However, treatment significantly reduced both aldehydic and ketonic derivatives of oxidatively modified proteins (OMPs), decreasing them from 28.82 ± 7.56 to 12.66 ± 2.24 nmol·mg^{-1} protein (-56.1%; $p < 0.001$) and from 35.01 ± 9.26 to 16.15 ± 2.68 nmol·mg^{-1} protein (-53.9%; $p < 0.001$), respectively. These results suggest that chloramine-T can substantially suppress protein oxidation processes and limit secondary oxidative damage to cellular macromolecules.

There were no significant differences in the activities of the key antioxidant enzymes superoxide dismutase (SOD) (432.03 ± 103.37 vs. 410.62 ± 129.79 U·mg^{-1} protein, $p = 0.69$), catalase (CAT) (0.44 ± 0.37 vs. 0.32 ± 0.21 U·mg^{-1} protein, $p = 0.39$) and glutathione reductase (GR) (2.68 ± 1.17 vs. 2.54 ± 1.07 U·mg^{-1} protein, $p = 0.79$). Similarly, GPx activity decreased slightly by 20.1% (from 67.14 ± 62.72 to 53.66 ± 30.54 U·mg^{-1} protein, $p = 0.55$) without reaching statistical significance. Conversely, total antioxidant capacity (TAC) increased by 18.8% in treated fish (from 27.25 ± 12.18% to 32.36 ± 17.57%, $p = 0.46$). Thus, exposure to chloramine-T did not significantly affect the enzymatic antioxidant system, but markedly decreased markers of protein oxidative damage, suggesting protective modulation of the tissue-level oxidative balance (Table 1).

Table 1

Activities of antioxidant enzymes in the cardiac tissue of rainbow trout bathed in chloramine-T.

Parameters / groups	SOD, U·mg⁻¹ protein	CAT, U·mg⁻¹ protein	GR, U·mg⁻¹ protein	GPx, U·mg⁻¹ protein
Untreated control group	432.03 ± 103.37	0.44 ± 0.37	2.68 ± 1.17	67.14 ± 62.72
Chloramine-T group	410.62 ± 129.79	0.32 ± 0.21	2.54 ± 1.07	53.66 ± 30.54

To better understand these changes, effect size and correlation analyses were performed. Exposure to Chloramine-T significantly decreased lipid peroxidation ($p = 0.000055$; Cohen's $d = 2.90$) and aldehydic OMP ($p = 0.000083$; Cohen's $d = 2.77$), corresponding to reductions of approximately 46% and 50%, respectively. The effect size for ketonic OMP was small and not significant ($p = 0.688$; $d = 0.18$), indicating the selective suppression of aldehydic protein oxidation products. SOD activity showed a mild, non-significant tendency to increase (Cohen's $d = 0.40$), whereas CAT activity remained almost unchanged (Cohen's $d = 0.12$). This confirms limited enzyme-dependent adaptation.

Correlation analysis revealed a very strong positive association between TBARS and aldehydic derivatives of OMP ($r = 0.99$, $p < 0.001$), indicating a close relationship between lipid and protein oxidation. TBARS also exhibited moderate positive correlations with GR activity ($r = 0.38$) and SOD ($r = 0.37$), suggesting the activation of compensatory enzymatic mechanisms in response to oxidative stress. However, no meaningful correlations were found between CAT and SOD activity ($r = -0.10$), or between TAC and other antioxidant parameters. These results suggest that the non-enzymatic antioxidant reserve may not be the primary factor in modulating oxidative stress under short-term exposure to chloramine-T.

A multiple linear regression model, with antioxidant enzymes (SOD, CAT, GR, GPx and TAC) as the predictors, explained 48% of the variability in TBARS levels ($R^2 = 0.48$). GR activity contributed most strongly to this relationship ($\beta = 0.36$, $p = 0.04$), followed by SOD ($\beta = 0.31$, $p = 0.08$). This suggests that glutathione-dependent enzymes, particularly GR, play a key compensatory role in maintaining lipid redox balance during oxidative challenges induced by chloramine-T.

Overall, the findings demonstrate that chloramine-T treatment markedly reduces the oxidative modification of lipids and proteins in cardiac tissue without significantly affecting the components of the enzymatic antioxidant defence system. The strong TBARS–OMP correlation highlights a shared pathway of oxidative injury, whereas the regression data emphasise the pivotal role of the glutathione redox cycle in sustaining cardiac antioxidant homeostasis during exposure.

Discussion. This study sheds new light on the oxidative and antioxidant responses of rainbow trout (*Oncorhynchus mykiss*) cardiac tissue following exposure to chloramine-T baths, which are commonly used in aquaculture as a disinfectant to control bacterial and parasitic infections. The results indicate that, while inducing measurable oxidative responses, chloramine-T selectively decreases protein oxidation, leaving most enzymatic antioxidant activities largely unaffected. This dual pattern suggests an adaptive balance between oxidative stress and antioxidant defences in fish cardiac tissue. These findings are particularly relevant given the increasing reliance on chemical disinfection protocols in intensive aquaculture, where repeated low-dose exposure may have cumulative or

hormetic effects (Tkachenko H. et al., 2013). It is crucial to understand these biochemical responses in order to evaluate the trade-off between the efficacy of disinfection and the potential for sublethal physiological stress in farmed fish.

A significant finding of this study was the substantial decrease in aldehydic and ketonic derivatives of oxidatively modified proteins (OMPs), which fell by over 50% following exposure to chloramine-T. These observations suggest a potential protective modulation of protein oxidation processes, possibly through reduced reactive oxygen species (ROS) generation or enhanced protein repair and degradation systems. A similar decline in protein carbonyls has been reported in studies where moderate oxidative challenges have triggered compensatory mechanisms that upregulate proteostasis pathways (Tkachenko H. et al., 2021). The decrease in oxidative protein damage, accompanied by a mild and statistically insignificant 23.6% reduction in lipid peroxidation, implies that short-term exposure to chloramine-T does not cause overt oxidative stress, but instead initiates redox-regulatory adjustments aimed at maintaining cellular integrity. This is consistent with recent findings in zebrafish and carp, where sublethal exposure to oxidants triggered the expression of protective antioxidant genes without inducing cytotoxicity (Zhao X. et al., 2016; Saputra F. et al., 2024; Lai Z. and He M., 2025; Qiu M. et al., 2025). This mild oxidative preconditioning effect could be an initial stage in the adaptation of aquatic organisms to stress.

Interestingly, there were no statistically significant changes in the activity of the antioxidant defence system's enzymatic components: superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx). The modest increase in SOD (Cohen's $d = 0.40$) and stability of CAT (Cohen's $d = 0.12$) suggest that the antioxidant system remained stable during exposure. This finding contrasts with several studies that have reported elevated SOD and CAT activities in response to more severe oxidative challenges (Liu, Y. et al., 2024; Zulfahmi I. et al., 2025). This suggests that the oxidative burden induced by chloramine-T was moderate and within the adaptive capacity of cardiac cells. The 18.8% increase in total antioxidant capacity (TAC) may reflect the transient activation of low-molecular-weight antioxidants such as glutathione and ascorbate. However, as this increase was not statistically significant, it implies that non-enzymatic antioxidants played only a secondary role under the studied conditions. Further investigation into the transcriptional regulation of antioxidant enzymes and non-enzymatic scavengers could clarify the molecular basis of this stability. Future transcriptomic or proteomic analyses could clarify whether this apparent biochemical stability is due to redox-related genes or post-translational enzyme modifications.

Correlation and regression analyses provided additional insight into the mechanisms of oxidative regulation. The strong positive correlation between TBARS and aldehydic OMP ($r = 0.99$, $p < 0.001$) indicates close interaction between lipid and protein oxidation pathways, consistent with the hypothesis that lipid peroxidation products can induce protein carbonylation through secondary radical interactions (Dalle-Donne et al., 2003). Moderate correlations between TBARS and both SOD ($r = 0.37$) and GR ($r = 0.38$) imply that these enzymes could be involved in a compensatory response to lipid peroxidation, modulating the superoxide and glutathione redox balances respectively. The multiple linear regression model explained 48% of the variance in TBARS levels ($R^2 = 0.48$) and identified GR as the most influential enzymatic predictor ($\beta = 0.36$, $p = 0.04$). This emphasises the central role of glutathione-dependent mechanisms in maintaining lipid redox homeostasis. This is consistent with previous findings that GR and the glutathione cycle play a key role in determining cardiac resilience to oxidative stress (Hellou J. et al., 2012; Srikanth K. et al., 2013). These results support the use of GR

activity as a biomarker for assessing cardiac oxidative status in fish exposed to environmental or pharmacological stressors. Therefore, GR activity could be used as an indicator of disturbances to the redox balance in fish exposed to environmental or chemical oxidants.

The selective attenuation of protein oxidation, accompanied by stable enzymatic antioxidants, can be interpreted as an adaptive phenomenon characteristic of hormesis. In this process, low-level exposure to oxidants triggers protective redox signalling rather than overt oxidative injury. As a mild oxidising agent, chloramine-T may have induced transient redox signalling pathways that modulate the thiol-based regulation of cellular proteins, without overwhelming the antioxidant defences. Similar effects to those of preconditioning have been observed in aquatic organisms exposed to sublethal oxidative stressors, resulting in increased resilience and maintenance of tissue redox equilibrium (Sen C. K., 1998; Anraku M. et al., 2003; Hermes-Lima M. et al., 2015). From an applied perspective, understanding such hormetic responses could contribute to the development of redox-based strategies to enhance the robustness of fish in aquaculture. This response could be exploited to improve stress tolerance in aquaculture, provided exposure levels are carefully controlled and monitored.

Rivero-Wendt C. L. G. et al. (2023a) conducted a study to evaluate the cytogenotoxic and toxic effects of exposure to chloramine-T (CL-T) in adult zebrafish (*Danio rerio*). Male and female fish were exposed to CL-T concentrations of 70, 140 and 200 mg/L for 96 hours. After this period, biomarkers of nuclear damage, histopathological alterations and acetylcholinesterase (AChE) activity were analysed. Significant increases in nuclear abnormalities and distinct optical density profiles of erythrocyte nuclei were observed, suggesting the potential cytogenotoxicity of CL-T, particularly at higher concentrations. Histopathological analyses of gill and liver tissues revealed low to moderate toxicity, while AChE activity remained unaffected. The authors emphasised that these cytogenotoxic alterations, even in the absence of overt neurotoxicity, may impair cellular homeostasis and long-term fish health. This is particularly relevant for aquaculture systems involving repeated or chronic exposure to disinfectants. These results suggest that despite its widespread use as a disinfectant in aquaculture, chloramine-T may induce sublethal genotoxic and tissue-level effects in fish. Alongside the current findings relating to rainbow trout, these results imply that the genotoxic and oxidative effects of chloramine-T may differ depending on the type of tissue, the developmental stage and the intensity of exposure.

In a related study, Rivero-Wendt et al. (2023b) investigated the toxicological effects of chloramine-T (CL-T) on *Danio rerio* embryos. The study focused on lethality, developmental abnormalities and AChE activity. The embryos were exposed to CL-T concentrations ranging from 16 to 256 mg/L for 96 hours to determine the LC_{50} values and assess sublethal outcomes. The LC_{50} values were found to be 143.05 ± 3.11 mg/L after 24 hours and 130.97 ± 7.4 mg/L after 96 hours, indicating a dose-dependent toxic response. Exposure to CL-T resulted in delayed hatching, a reduced heart rate, cardiac oedema and a loss of equilibrium in the larvae, as well as significant AChE inhibition at concentrations of 64 and 128 mg/L. Together with the findings in adult zebrafish, these data demonstrate that CL-T can exert developmental, genotoxic and neurotoxic effects across the life stages of fish. This underlines the importance of carefully evaluating its concentration and exposure duration in aquaculture practices. These findings show that chloramine-T causes cardiotoxic and neurotoxic effects during the early stages of zebrafish development. This raises concerns about its safety in aquaculture environments. Therefore, a comprehensive risk assessment of chloramine-T in aquaculture requires the

integration of biochemical, developmental and histological endpoints across different model species.

In summary, this study's findings demonstrate that exposure to chloramine-T in rainbow trout hearts leads to a significant reduction in oxidative protein damage without disrupting enzymatic antioxidant defences. The strong correlation between lipid and protein oxidation, alongside the significant role of glutathione reductase in regulating TBARS variability, highlights the importance of the glutathione redox cycle in maintaining cardiac oxidative homeostasis. These results improve our understanding of the biochemical adaptations of fish cardiac tissue to disinfectant-induced oxidative stimuli, suggesting that moderate exposure to chloramine-T activates protective redox mechanisms rather than causing oxidative injury. However, when considered alongside evidence of genotoxicity and developmental toxicity in zebrafish, the findings highlight the importance of balancing the antimicrobial benefits of chloramine-T with its potential biological risks. Future studies should examine long-term and repeated exposure scenarios, as well as making cross-tissue comparisons, in order to fully evaluate the systemic impact of chloramine-T in aquaculture settings.

Conclusions. The present study demonstrates that short-term exposure to chloramine-T baths induces a selective oxidative response in the heart tissue of rainbow trout. Despite a mild decrease in lipid peroxidation, chloramine-T caused a significant reduction in the levels of aldehydic and ketonic derivatives of oxidatively modified proteins, indicating strong suppression of oxidative damage to proteins. This suggests that chloramine-T does not trigger oxidative injury at the cardiac level under the tested conditions, but may instead promote redox adaptation through modulation of protein oxidation pathways.

The activities of key antioxidant enzymes (SOD, CAT, GR and GPx) remained stable, confirming that the enzymatic antioxidant system was not overwhelmed by the oxidative challenge. Correlation and regression analyses revealed that glutathione reductase activity was the strongest predictor of lipid peroxidation intensity, highlighting the central role of the glutathione redox cycle in maintaining lipid homeostasis. The strong correlation between lipid and protein oxidation (TBARS-OMP) highlights the interconnectedness of oxidative processes that target different macromolecules within cardiac cells.

Taken together, these findings suggest that, when used within the recommended concentration range, chloramine-T does not induce harmful oxidative stress in the cardiac tissue of rainbow trout. Instead, it may activate subtle adaptive mechanisms that preserve redox equilibrium. From an aquaculture perspective, this supports the safe therapeutic application of chloramine-T, provided exposure time and dosage are carefully controlled. Future studies should extend these observations to chronic or repeated exposure models and evaluate transcriptomic and proteomic markers of oxidative signalling in order to fully elucidate the molecular mechanisms underlying the observed redox modulation.

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