Triphenyltin induced oxidative damage in sea urchin *Glyptocidaris crenularis*

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ABSTRACT

Triphenyltin (TPT), a widely distributed organotin compound, presents considerable environmental risks, especially within aquatic ecosystems. To assess the repercussions of TPT exposure on oxidative status indicators in sea urchin *Glyptocidaris crenularis*, we examined malondialdehyde (MDA) levels, total antioxidative capacity (T-AOC), and the activities of pivotal antioxidative enzymes. Results revealed significant lipid peroxidation, evidenced by elevated MDA levels in *G. crenularis*. Concurrently, T-AOC was markedly compromised, implying depletion in the capacity of G. crenularis to scavenge free radicals. The activities of Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione peroxidase (GPX) were notably modified following exposure to TPT in *G. crenularis*, indicating that oxidative injury was induced by TPT. GPX activity was increased at higher TPT concentration groups. Meanwhile, SOD and CAT activities decreased across all TPT exposure levels, underscoring a diminished detoxification capacity in *G. crenularis*. These findings suggest that TPT induces oxidative damage in *G. crenularis*, disrupting lipid integrity and impairing the antioxidative defense mechanisms.

Keywords: Sea urchin, organotin, superoxide dismutase, catalase, lipid peroxidation

1. INTRODUCTION

Triphenyltin (TPT) is a pivotal component of antifouling paints in the maritime industry, utilized to prevent the buildup of fouling organisms, particularly barnacles, on ship hulls[1]. Additionally, TPT finds extensive application as a biocide in both agriculture and industry[1], especially in pesticides and wood preservatives[2]. Despite its widespread use, TPT ranks among the most hazardous anthropogenic pollutants in aquatic ecosystems, posing severe risks to aquatic organisms[2]. TPT functions as a potent endocrine disruptor[3,4], presenting a significant threat to non-target marine organisms[5]. Even at remarkably low concentrations of 1-10 ng/L tin, TPT can bioaccumulate in aquatic organisms[6,7], leading to developmental malformations[3,8]. Consequently, TPT undermines the growth[9], development[8,10], and even viability[11,12] of non-target organisms in aquatic ecosystems. Numerous countries have enacted regulations to control or ban the use of TPT in antifouling paints[2]. Nevertheless, TPT continues to be utilized as a biocide in various other sectors, including material and wood preservation[2].

Aquatic organisms are inherently exposed to various environmental pollutants[13], including organic chemicals[14] (such as plasticizers[5], pesticides[15], and organotins[1]), heavy metals (like copper[16]), and microplastics[17]. Current evidence indicates that environmental toxins are primary producers of reactive oxygen species (ROS), which damage DNA[18,19], as well as other macromolecules like lipids and proteins in aquatic animals[20]. Antioxidant defenses, including the activity of antioxidant enzymes, enable organisms to adapt to elevated ROS production[21]. Oxidative stress arises when the balance between prooxidants and antioxidants shifts in favor of oxidants under environmental stress[2]. Consequently, the disruption of normal cellular functions by oxidative damage results in cell death via apoptosis and necrosis[22]. Most studies employing antioxidant enzymes (e.g., catalase [CAT], glutathione peroxidase [GPX], superoxide dismutase [SOD]) as biomarkers have been carried out in fish[23] and marine invertebrates[4].

The sea urchin has emerged as a model organism in marine toxicology owing to its ecological significance, transparent embryos, and heightened sensitivity to environmental pollutants[4]. These characteristics render the sea urchin an ideal subject for investigating the toxicological effects of environmental pollutants, including microplastics[17] and heavy metals[24]. Oxidative stress manifests in sea urchins but is also influenced by chemical exposure[1], temperature

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fluctuations[25], and other environmental stressors[26]. However, TPT-induced oxidative stress and its impacts on the antioxidative defense system in the sea urchin *Glyptocidaris crenularis* remain inadequately understood.

This study aims to elucidate the toxic effects of TPT on *G. crenularis* by analyzing lipid peroxidation, total antioxidative capacity (T-AOC), and activities of antioxidative enzymes (SOD, GPX, and CAT). *G. crenularis* were exposed to a range of TPT concentrations (0, 5.6, 7.5, 10, 13.5, and 18 μg/L) for 48 hours to evaluate oxidative stress-related biochemical indicators. The findings are expected to provide an understanding of the biochemical mechanisms by which TPT exerts its toxic effects on sea urchins.

2. MATERIALS AND METHODS

2.1 Materials

G. crenularis, with a shell diameter of (6.0 ± 0.4) cm, was procured from Changxing Seafood Market in Dalian, China. *G. crenularis* were acclimatized for 7 days in a hydrostatic culture system with filtered natural seawater maintained at 22 ± 1°C, pH 7.8 ± 0.5, and salinity 32.2 ± 0.3 ppm, under a photoperiod of 12 h light: 12 h dark. *G. crenularis* was fed once a day with fresh *Undaria pinnatifida*.

TPT chloride (purity ≥ 98%) and acetone were obtained from TCL (Shanghai, China) Chemical Industry Development Co., Ltd. Acetone was employed as a co-solvent for TPT. Protein, T-AOC, malondialdehyde (MDA), CAT, SOD, and GPX levels were quantified using commercial assay kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2 Experimental design

Six TPT concentrations were established: 0, 5.6, 7.5, 10, 13.5, and 18 μg/L. Four replicates were prepared for each group, with laboratory sand-filtered seawater serving as the control. The experiment was conducted in 10-liter plastic buckets, each containing 20 sea urchins. The test solution was replaced every 24 hours throughout the experimental period. *G. crenularis* activity and mortality were observed and recorded daily, with deceased individuals promptly removed. A 1 ml sterilized syringe was obliquely inserted through the peritoneum into the body cavity of *G. crenularis* to extract body fluids. The body fluid was centrifuged at 6000 rpm for 15 minutes at 4 °C, and the supernatant was stored in an ultra-low temperature freezer (-80 °C). The supernatant was used for the quantification of protein, MDA, T-AOC, CAT, GPX and SOD.

2.3 Oxidative damage indicators

All parameter related optical density values were determined using a microplate reader (Molecular Devices, Sunnyvale, CA, USA) and spectrophotometer (Thermo Fisher Instruments Inc., Vantaa, Finland). The soluble protein content of *G. crenularis* was determined using the Bradford method, with a protein standard solution concentration of 0.563 g/L employed as a control. The OD values of the reaction mixtures were measured using an enzyme marker at 595 nm. The T-AOC was determined by the Fe^{3+}/Fe^{2+} reduction method, with the measured values expressed as U/mg protein. MDA content was quantified using the thiobarbituric acid reactive substances assay, with the OD value of the product measured at 532 nm.

The results of the CAT activity assay are expressed in units per milligram of protein (U/mg protein), where one CAT unit is defined as the enzyme activity that decomposes 1 mmol of H_2O_2 per second. SOD activity was assessed using a method based on the inhibition of cytochrome c reduction. The extent of cytochrome c reduction by superoxide anions was quantified by measuring the increase in absorbance at 550 nm. GPX activity was determined by measuring the reduction of oxidized glutathione at a wavelength of 405 nm.

2.4 Statistical analysis

Statistical analyses were conducted using GraphPad Prism 8, with results presented as mean ± standard deviation. The significance of each parameter was evaluated using one-way ANOVA. The P value for the experimental group exposed to TPT compared to the control group was less than 0.05 (* indicates a significant difference), and ** indicates a highly significant difference ($P < 0.01$).

3. RESULTS

3.1 Triphenyltin-induced lipid peroxidation in *Glyptocidaris crenularis*

At a TPT concentration of 5.6 μg/L, the MDA content in *G. crenularis* was slightly lower than that of the control group. With increasing TPT exposure levels, the MDA content in *G. crenularis* in the other TPT-treated groups exceeded that of the control group. At TPT concentrations of 7.5, 10, and 13.5 μg/L, the MDA content in *G. crenularis* was significantly higher than that of the control group ($P < 0.01$). The MDA content in *G. crenularis* peaked at a concentration of 10 μg/L compared to the control group and was slightly higher at a concentration of 18 μg/L (Figure 1).

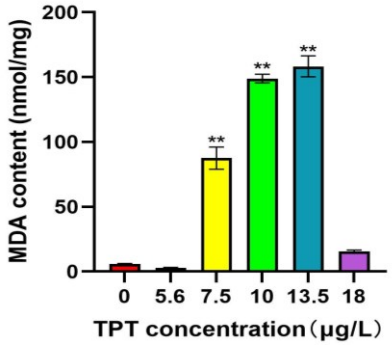

Figure 1. Triphenyltin induced lipid peroxdation in *Glyptocidaris crenularis.*

3.2 Triphenyltin induced change of the total antioxidant capacity in *Glyptocidaris crenularis*

The T-AOC values in *G. crenularis* exposed to 10 μg/L TPT were slightly higher than those in the control group. With increasing TPT exposure levels, the T-AOC values in *G. crenularis* in the other groups were significantly lower than those in the control group. At a TPT concentration of 7.5 μg/L, the T-AOC was significantly lower than that in the control group ($P < 0.05$). At TPT concentrations of 5.6, 13.5, and 18 μg/L, T-AOC values showed a highly significant decrease compared to the control group, with the lowest T-AOC value observed at 18 μg/L ($P < 0.01$, Figure 2).

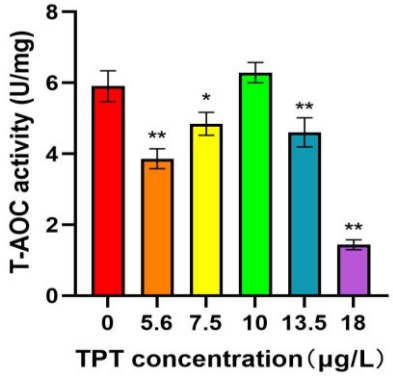

Figure 2. Triphenyltin induced change of the total antioxidant capacity in *Glyptocidaris crenularis.*

3.3 Triphenyltin exposure influences antioxidant enzyme activity in *Glyptocidaris crenularis*

At TPT concentrations of 5.6 and 7.5 μg/L, SOD activity in *G. crenularis* was significantly lower than that in the control group ($P < 0.01$). At a TPT concentration of 10 μg/L, SOD activity in *G. crenularis* was slightly higher than that in the control group. At a TPT concentration of 13.5 μg/L, SOD activity was slightly lower than in the control group, while at 18 μg/L, SOD activity in *G. crenularis* was significantly lower than that in the control group ($P < 0.05$, Figure 3A).

The activity of GPX in *G. crenularis* exhibited a general trend of initially decreasing and then increasing with increasing TPT exposure levels. GPX activity was significantly reduced at a TPT concentration of 5.6 μg/L ($P < 0.05$). At 7.5 μg/L TPT concentration, sea urchin GPX activity was slightly lower than that of the control group. However, at TPT concentrations of 10, 13.5, and 18 μg/L, GPX activity was significantly higher than in the control group ($P < 0.01$), with GPX activity in *G. crenularis* peaking at the concentration of 18 μg/L (Figure 3B).

Across all levels of TPT exposure, CAT activities in *G. crenularis* exhibited a consistent decrement compared to the control group. CAT activities in *G. crenularis* notably decreased across TPT concentrations ranging from 5.6 to 18 μg/L, with the most significant decline at 7.5 μg/L TPT treated Group ($P < 0.01$, Figure 3C).

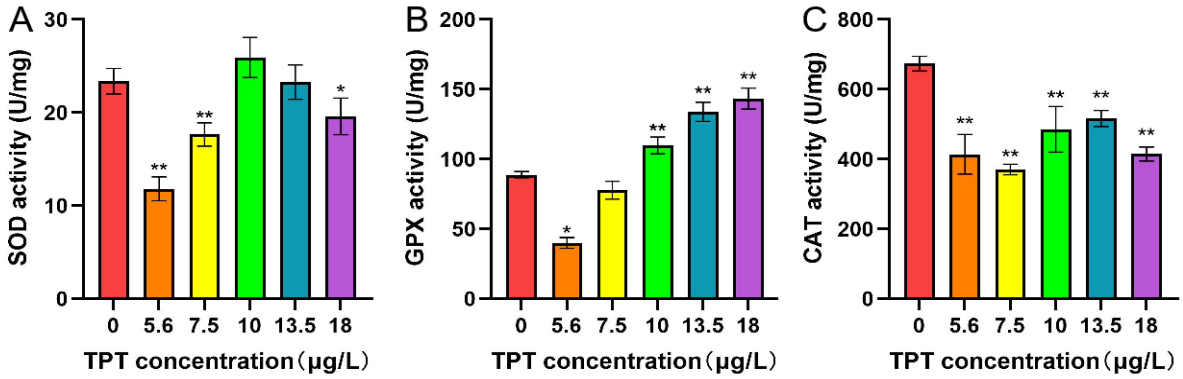

Figure 3. Triphenyltin exposure influences antioxidant enzyme activity in *Glyptocidaris crenularis*. (A): SOD; (B): GPX; (C): CAT.

4. DISCUSSION

TPT is often integrated into pesticides and marine antifouling paints, potentially resulting in its introduction into the aquatic environment, posing a considerable threat to non-target organisms[8]. This threat manifests in various ways, including developmental malformations in zebrafish embryos and larvae[8], as well as disruptions to the endocrine system and cellular functions[3]. Given the widespread use of TPT in various industrial applications and its potential to leach into aquatic environments, understanding its impact on aquatic organisms is crucial. Oxidative stress, arising from an imbalance between the generation of ROS and the organism's antioxidative defense systems' capacity, represents a pivotal mechanism of toxicity capable of inflicting cellular and molecular harm[27]. Exposure to 10 μg/L TPT for 14 days induced oxidative stress in zebrafish larvae[23]. Exposure to 10 ng/L TPT triggered oxidative stress and significantly diminished antioxidant enzyme activity in goldfish[27]. Exposure of *Chlorella sp.* to 200 ng/L TPT for 12 days initiated the onset of oxidative damage[28].

The present study delved into the toxicological ramifications of TPT on *G. crenularis* by scrutinizing lipid peroxidation, T-AOC, and the activities of antioxidative enzymes. Lipid peroxidation stands as a pivotal gauge of oxidative harm inflicted upon cellular membranes[28], whereas T-AOC offers a comprehensive assessment of the organism's capacity to combat oxidative stress[29]. Our findings unveil a notable surge in lipid peroxidation levels among TPT-exposed *G. crenularis* specimens (Figure 1). The discerned rise in MDA levels implies that TPT exposure induced oxidative stress in *G. crenularis*, culminating in membrane deterioration and compromised cellular functions. Upon TPT exposure, a decline in T-AOC was noted in *G. crenularis* specimens (Figure 2). This compromised T-AOC could potentially render *G. crenularis* more vulnerable to oxidative harm, thereby exacerbating the detrimental consequences of TPT exposure on cellular equilibrium. These findings corroborate prior research indicating the pro-oxidative impacts of TPT across diverse aquatic species[10].

Antioxidant enzymes, including GPX, SOD, and CAT, constitute vital elements of an aquatic organism's internal defense system, synergistically tasked with neutralizing deleterious ROS[2,30]. Following exposure to TPT, SOD activities in *G. crenularis* demonstrated a downward trajectory (Figure 3A), indicative of a compromised capacity to scavenge superoxide radicals. A conspicuous modulation in GPX activities was discerned in TPT-exposed *G. crenularis* (Figure 3B), highlighting the pivotal role of GPX in the detoxification of H_2O_2 and lipid hydroperoxides[30]. The decrease in GPX activities could exacerbate oxidative stress by hampering the elimination of lipid hydroperoxides, consequently perpetuating lipid peroxidation and cellular damage in *G. crenularis*. The findings revealed a marked decrease in CAT activities across all TPT exposure levels in *G. crenularis*, signaling impaired H_2O_2 detoxification capability (Figure 3C). The interdependence between lipid peroxidation and the activities of SOD, GPX, and CAT underscores the complex interplay between oxidative stress and antioxidant defense mechanisms triggered by TPT exposure in *G. crenularis*. The noted elevation in lipid peroxidation could stem from the imbalance between ROS production and the antioxidant defense mediated by SOD, CAT, and GPX, culminating in oxidative damage[3].

5. CONCLUSIONS

Through elucidating the repercussions of TPT exposure on lipid peroxidation, T-AOC, and antioxidative enzyme activities in *G. crenularis*, our findings serve to underscore the inherent vulnerability of *G. crenularis* to TPT-induced oxidative stress, substantiated by the notable escalation in lipid peroxidation levels post-exposure. The discerned modifications in T-AOC and antioxidative enzyme activities, encompassing SOD, GPX, and CAT, serve to further accentuate the intricate interplay between oxidative stress and the antioxidant defense system in *G. crenularis*.

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