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Protective Action of Hydrolysable Tannins Against Bisphenol A Toxicity on Zebrafish Development

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Abstract

Hydrolysable tannins (HTs) are polyphenolic compounds extracted from plants consisting of a sugar core, esterified with phenolic acids, such as gallic or ellagic acid. These phenolic acids are responsible for their well-known antioxidant, anti-tumor, antimicrobial, and anti-inflammatory properties. This study investigated the potential protective role of HTs against bisphenol A (BPA), an environmental pollutant known to have toxic effects. Zebrafish embryos were exposed to BPA at 25.0 μM alone and in combination with HTs at 5.0, 10.0, and 20.0 μgL^{-1} for 72 h. The results showed that HTs at 20.0 μgL^{-1} improved hatching and heart rate affected by BPA and reduced the phenotypic alterations caused by BPA. In addition, molecular analysis of genes involved in development showed that the down-regulation of *cd63*, *zhe1*, *klf4*, *hand2*, *sox9b*, and *gata4* genes in the BPA group were improved with HTs 20.0 μgL^{-1}. Furthermore, HTs were able to reduce the increased lipid content caused by exposure to BPA. These results demonstrate that HTs have a protective effect on the development of zebrafish exposed to BPA, suggesting that they could potentially exert protective effects in response to other environmental stressors.

Keywords: hydrolysable tannins; bisphenol A; zebrafish; embryos; toxicity; protective action

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1. Introduction

The vulnerability of the body to environmental stress and diseases caused by toxic agents, such as heavy metals and persistent organic pollutants (POPs), could be reduced by a healthy lifestyle and a diet rich in polyphenols [1–3]. Polyphenolic compounds originate from the secondary metabolism of plants and exhibit significant anti-inflammatory and antioxidant properties [4]. Among polyphenolic compounds, tannins are particularly important because they are present in a wide variety of plant-based foods and are essential components of the human diet. They are found in the roots, stems, seeds, fruits, and leaves of plants and play a protective role against infections, insects, and herbivorous predators [5,6].

Tannins are classified based on their chemical structure into phlorotannins, condensed tannins, and hydrolysable tannins. Phlorotannins (PTs) are oligomers or polymers of phloroglucinol-1,3,5-trihydroxybenzene, found exclusively in brown algae [7,8]. Condensed tannins are also polymers or oligomers but consist of flavan-3-ol units and they are found in green tea, aronia berries, apples, hazelnuts, and sorghum [8,9]. Hydrolysable tannins are characterized by a central sugar core, most commonly D-glucose, in which the hydroxyl groups are esterified with phenolic acids. Depending on whether gallic acid or ellagic acid is involved in their structure, the resulting compounds are classified as gallotannins

or ellagitannins, respectively [10]. Hydrolysable tannins are present in pomegranates, mangoes, guavas, raspberries, and chestnuts [9]. Hydrolysable tannins can be metabolized, releasing phenolic acids, which demonstrate strong antioxidant properties due to their abundance of hydroxyl groups [11]. In addition, the combination of hydrolysable tannins with condensed tannins results in the formation of hybrid structures known as complex tannins [11]. Tannins can protect against various biotic and abiotic stress factors [9]. Several studies have attributed anti-inflammatory, anti-tumor, antimicrobial, and antiviral effects to hydrolysable tannins in human and animal health [12–14]. In particular, they can counteract oxidative stress and pro-inflammatory responses induced by contaminants such as heavy metals and POPs thanks to their transition metal chelation process and stimulated enzymatic system [15].

Among POPs, bisphenol A (BPA) has been used for years in the production of polycarbonate plastics and epoxy resins. These materials are used to produce food and non-food objects. Examples of food objects include disposable tableware and food packaging. Non-food objects include thermal paper, car parts, and some medical devices [16]. Given its widespread use, humans are inevitably exposed to BPA. Its presence in the environment results from leaks from landfills, open burning of waste, and natural decomposition of plastics [17]. It has been widely demonstrated that BPA is an endocrine disruptor, capable of binding primarily to α and β estrogen receptors (ERs) with a greater affinity for β receptors due to its similar chemical structure to synthetic non-steroid estrogen diethylstilbestrol [15,18]. Its presence has been linked to breast and testicular cancer, cardiovascular diseases, polycystic ovary syndrome, diabetes, autism spectrum disorders, and thyroid dysfunction [18–20]. In addition, bisphenol A is also considered an obesogenic substance capable of interfering with lipid metabolism and promoting adipogenesis through the alteration of hormonal and genetic regulatory pathways [21]. Although the use of bisphenol A in food-contact materials has been prohibited by the European Food Safety Authority (EFSA), residual exposure may still occur during the transitional phase, as manufacturers are permitted to exhaust existing stocks and progressively adjust their production processes to comply with the new legal requirements [22].

Based on previous evidence of the antioxidant and anti-inflammatory properties of hydrolysable tannins and on the proven toxicity of bisphenol A, this study aims to analyze whether HTs could counteract the toxic action of an environmental pollutant such as BPA. We used zebrafish embryos, a model organism widely used for its 70% genetic homology with humans, which is reflected in a similar response to toxic agents between the two organisms [23,24].

Zebrafish embryos were exposed to HTs 5.0, 10.0, and 20.0 μgL^{-1} in combination with BPA 25.0 μM. Hydrolysable tannins concentrations were selected from our previous study [25], in which low concentrations (5.0, 10.0, and 20.0 μgL^{-1}) were shown to not be toxic. Regarding BPA, the concentration used was based on previous studies [26,27]. Although it is higher than common environmental levels, which are around μgL^{-1}, the concentration of 25.0 μM (5707 μgL^{-1}) causes early effects that allow us to analyze the potential protective effects of hydrolysable tannins.

The parameters of toxicity were analyzed up to 72 h of treatment. The expression of genes involved in the hatching process (*cd63*, *zhe1*, and *klf4*) and in heart development (*hand2*, *sox9b*, and *gata4*) was evaluated through quantitative real-time PCR. In addition, because BPA exposure has been associated with the dysregulation of lipid metabolism [28], Nile red staining was performed to assess lipid accumulation. Nile red is a lipophilic fluorescent probe that selectively stains neutral lipid droplets, providing a qualitative and quantitative measure of lipid content [29]. Since BPA is an endocrine disruptor and an obesogenic substance, we expected to observe developmental alterations and changes in

lipid metabolism. Given that hydrolysable tannins are known to act as hormonal regulators and influence lipid metabolism, we hypothesize that HTs treatment could mitigate the toxic effects of BPA on embryonic development.

2. Materials and Methods

2.1. Preparation of Solutions

Hydrolysable gallotannins (Tan'Active GTC/E), extracted from Chinese gallnuts (*Rhus semialata*), were purchased by Silvateam S.p.a., (Cuneo, Italy), which declared a degree of tannin purity content equal to 96%, attributable to highly purified gallotannins [25]. Tannins were dissolved in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2 \cdot 2H_2O$, 0.33 mM $MgSO_4$) to reach the concentration to be tested (5.0, 10.0, and 20.0 μgL^{-1}).

Bisphenol A [2,2-Bis(4-hydroxyphenyl)propane, 4,4′-Isopropylidenediphenol] (Sigma-Aldrich, Saint-Quentin-Fallavie, France) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Milan, Italy). Then, BPA was diluted in E3 medium to achieve the concentration to be used (25.0 μM).

2.2. Zebrafish Embryos Collection

Wild-type AB zebrafish (female/male ratio of 2:1) were bred in the Facility of Biology Department of the University of Naples Federico II. They were housed in 20-L glass tanks with a photoperiod light/dark of 12 h:12 h, temperature of 28.0 ± 1.0 °C, pH 7.5 ± 0.3, and conductivity between 250 and 600 μS/cm, checked daily. They were fed with dry food (TetraMin Tropical Flake Fish®, Tetra, Blacksburg, VA, USA). The eggs were collected in the early morning, and then, the fertilized eggs were selected and rinsed with E3 medium.

2.3. Exposure Test

At 6 h post fertilization, after washing, embryos were exposed to BPA 25.0 μM alone and in combination with HTs at 5.0, 10.0, and 20.0 μgL^{-1} for 72 h. Additionally, embryos were exposed to HTs alone at the same concentrations. Two control groups were set up: one was exposed to E3 medium only (Ctrl), the other to DMSO at 0.01% to assess any potential effects of the solvent. A total of 20 embryos from each group were used. The experiments were performed in triplicate and followed National Italian (D.lgs 26/2014) and European (2010/63/EU) guidelines regarding the welfare of animals used for research purposes.

2.4. Analyses of Toxicity Parameters

Survival and hatching rates were registered at 24 h, 48 h, and 72 h of exposure and calculated by counting the number of live and hatched embryos on the total of embryos. Heart rate was analyzed at 72 h of treatment by observing the larvae under a light microscope and counting the heartbeat for 15 s and then calculated per minute [30].

2.5. Gene Expression Analysis

Total RNA extraction was carried out on 20 larvae per group at 72 h of exposure, using Direct-zol RNA Miniprep Plus Kit (ZYMO RESEARCH, Irvine, CA, USA). Spectrophotometric measurements (260/280 and 260/230 ratios) were used to evaluate quality and purity of RNA (Nanodrop spectrophotometer 2000 (Thermo Scientific Inc., Waltham, MA, USA). Then for cDNA synthesis, 1000 ng of RNA was retrotranscribed using All-In-One 5X RT MasterMix (Applied Biological Materials, Richmond, BC, Canada). For quantitative real-time PCR, 2 μL of cDNA was added to a reaction consisting of 0.5 μL of each primer (Table S1) at 10.0 μM and 10 μL of BlastTaqTM 2X qPCR MasterMix (Applied Biological Materials, Richmond, BC, Canada). The reaction was conducted in a StepOnePlus Real Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA) with these

conditions: 1 cycle for enzyme activation (95 °C for 3 min) and 40 cycles for denaturation and annealing/extension (95 °C for 15 s, 60 °C for 1 min). The melting curve analysis was performed according to the system instructions. *β-actin* was used as the endogenous gene to normalize the expression of *cd63*, *zhe1*, *klf4*, *hand2*, *sox9b*, and *gata4*. The normalization was calculated using the Ct value, through the REST software (Relative Expression Software Tool, version 1.9.12), based on Pfaffl's method [31].

2.6. Nile Red Staining

At 72 h of exposure, 10 larvae for each group were randomly selected for Nile red staining. Nile red was dissolved in acetone 0.4% to prepare a stock solution of 300 nM. The larvae were rinsed three times with E3 medium and then exposed to Nile red for 30 min in the dark at 28 °C. The larvae were rinsed again twice with E3 medium to remove the excess dye and observed through Axioskop 50 microscope (Carl Zeiss, Oberkochen, Germany) equipped with fluorescence, using 5× objective lens. Texas Red filter was used to detect Nile red fluorescence. The images were acquired by using Axiovision version 3.8 Software (Carl Zeiss). Semi-quantitative analysis of fluorescence intensity was performed through Image J version 1.54g on the binary image using the "threshold" tool and then "analyze particles" tool to count the fluorescent spot [32]. The data were reported in the graph as the ratio between treated and control.

2.7. Statistical Analysis

The experiments were performed in at least triplicate, and the data are presented as mean ± SEM. For the statistical analysis, GraphPad Prism Software (version 8.02 for Windows, GraphPad Software, La Jolla, CA, USA) was used. All groups were compared with each other through the one-way analysis of variance (ANOVA) method followed by Tukey's test (Table S2). In the gene expression analysis, Student's *t*-test was used to compare the BPA 25.0 μM group against the HTs 20.0 μgL^{-1} + BPA 25.0 μM group (Table S3).

3. Results

3.1. Toxicity Parameters

The results of hatching analysis revealed that embryos treated with HTs at 5.0, 10.0, and 20.0 μgL^{-1} showed a percentage comparable to the control group. In contrast, embryos exposed to BPA 25.0 μM showed a decrease in hatching at both 48 h and 72 h of exposure. The co-exposure with BPA + HTs at 5.0 μgL^{-1} and 10.0 μgL^{-1} did not improve the hatching process, unlike treatment with BPA 25.0 μM and HTs at 20.0 μgL^{-1}, where an improvement of the percentage was observed at 72 h of exposure. The group exposed to DMSO 0.01% was not affected by the treatment, confirming the absence of interference due to the solvent (Figure 1).

The heart rate measured at 72 h of exposure highlighted bradycardia in the group exposed only to BPA 25.0 μM. During co-exposure with 5.0 μgL^{-1} and 10.0 μgL^{-1} of HTs, no differences were found compared to BPA 25.0 μM alone, unlike co-exposure with 20.0 μgL^{-1} of HTs, where an improvement was observed. No differences emerged in the groups exposed to only HTs 20.0 μgL^{-1} and DMSO 0.01% compared to the control group (Figure 2).

At 72 h of treatment, phenotypic alterations were observed in the group exposed to BPA 25.0 μM. No significant differences were observed in the groups co-exposed to both HTs 5.0 μgL^{-1} + BPA 25.0 μM and HTs 10.0 μgL^{-1} + BPA 25.0 μM. In contrast, in the group co-exposed with HTs 20.0 μgL^{-1} + BPA 25.0 μM, a decrease in alterations was observed (Figure 3). The groups exposed to only HTs at 5.0 μgL^{-1}, 10.0 μgL^{-1}, and 20.0 μgL^{-1} did not show significant changes and were comparable to the control; therefore, they were not shown in the graph. Figure 3B,E showed pericardial edema in the group exposed to BPA

25.0 µM alone compared to the control group (Figure 3A,D), while Figure 3C,F showed a reduction in pericardial edema in the co-exposed group HTs 20.0 μgL^{-1} + BPA 25.0 µM. The group exposed to only HTs 20.0 μgL^{-1} and DMSO 0.01% have not reported any alterations, and the images were shown in Figure S1.

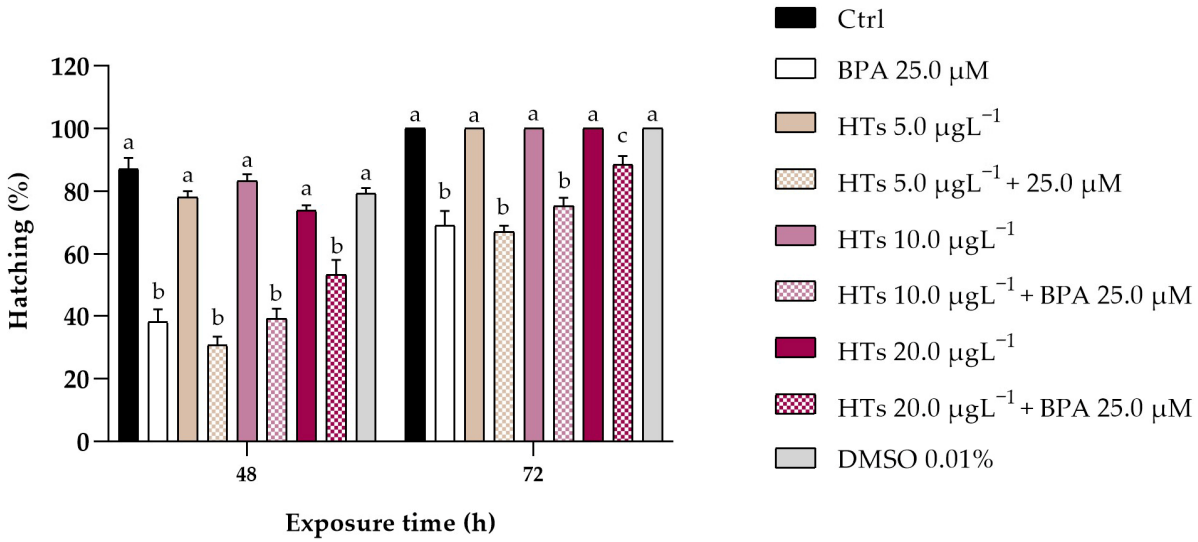

Figure 1. Hatching percentage at 24 h, 48 h, and 72 h of exposure (n = 3). One-way ANOVA followed by Tukey's test: different letters indicated significant differences between groups.

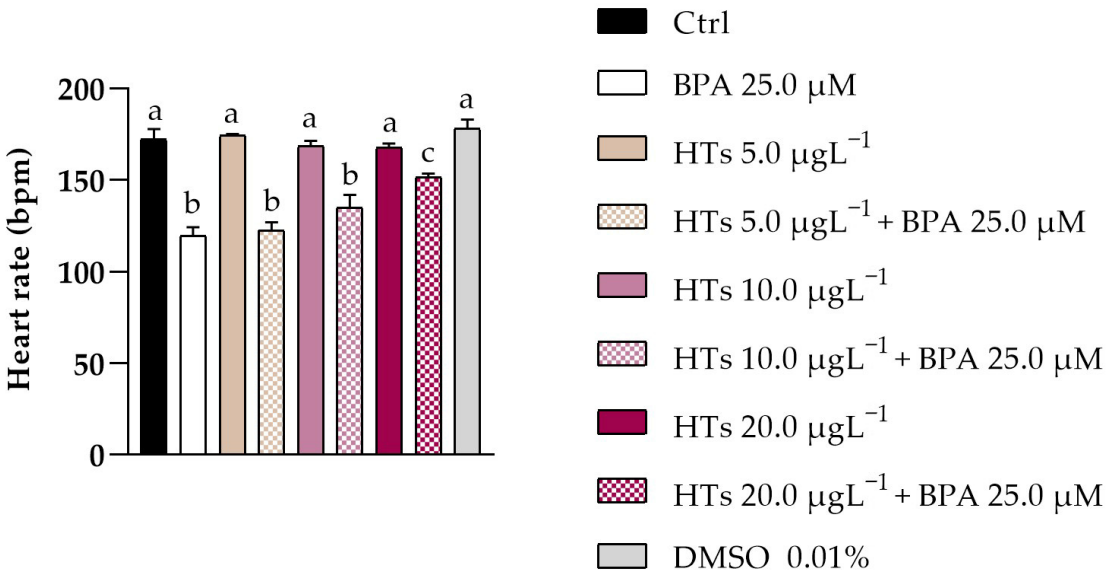

Figure 2. Heart rate at 72 h of exposure (n = 3). One-way ANOVA followed by Tukey's test: different letters indicated significant differences between groups.

3.2. Gene Expression

Analysis of gene expression were conducted only for the 20.0 μgL^{-1} HTs concentration because it was the only one capable of attenuating the toxic effect of BPA in the toxicity parameters. From the results, all genes were down-regulated in BPA 25.0 µM and HTs 20.0 μgL^{-1} + BPA 25.0 µM groups compared to the control group. However, in the co-exposure group, there was an improvement in down-regulation compared to the BPA group for all genes analyzed except for *sox9b*. In fact, only for *sox9b*, no significant differences emerged between BPA 25.0 µM and HTs 20.0 μgL^{-1} + BPA 25.0 µM (Figure 4). No alterations in the HTs 20.0 μgL^{-1} and DMSO 0.01% groups were observed.

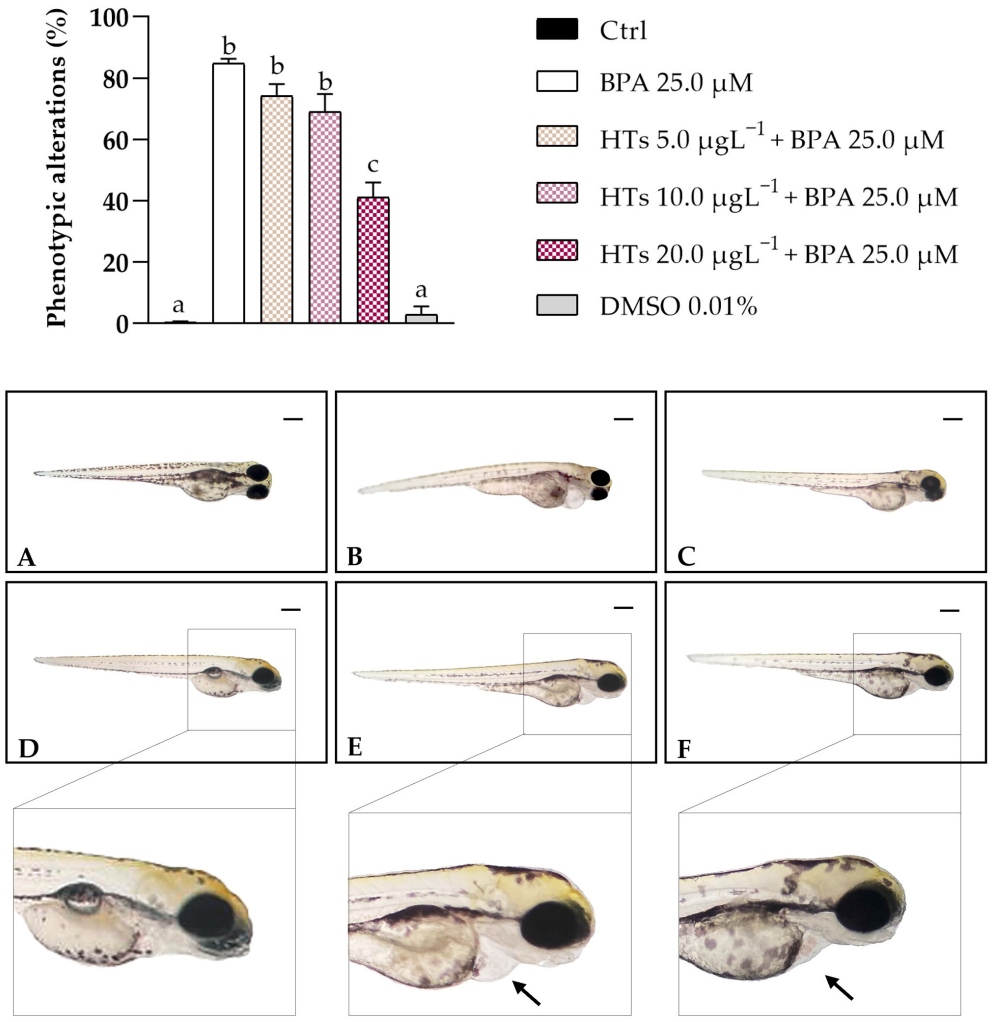

Figure 3. Phenotypic alterations at 72 h of exposure (n = 3). One-way ANOVA followed by Tukey's test: different lowercase letters indicated significant differences between groups. (**A**,**D**) Ctrl group; (**B**,**E**) BPA 25.0 µM group; (**C**,**F**) HTs 20.0 µgL^{-1} + BPA 25.0 µM group. Black arrows = pericardial edema. Scale bar: 450 µm.

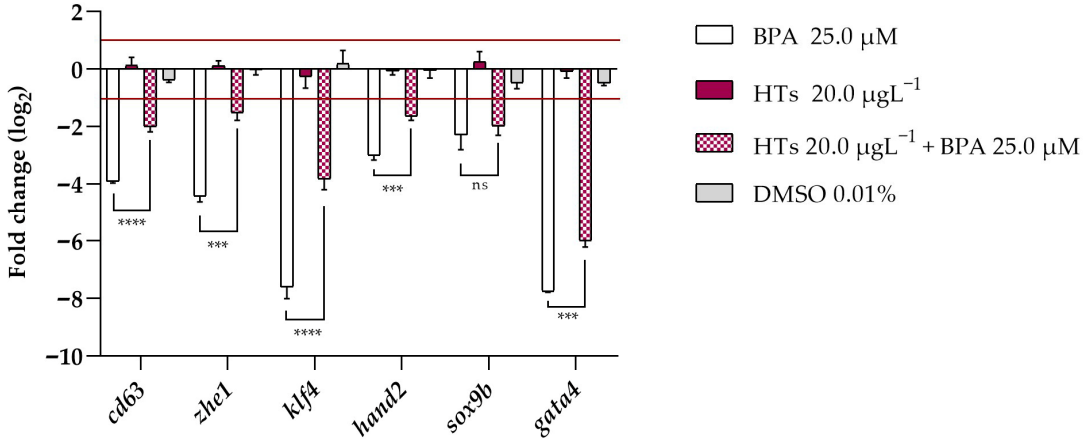

Figure 4. Gene expression analysis at 72 h of exposure (n = 4). Fold changes = $2^{-\Delta\Delta Ct}$. Red lines indicate fold change thresholds of 2 and 0.5, respectively. Values greater than 2 and less than 0.5 were considered significant compared to control. Significant differences between BPA 25.0 µM and HTs 20.0 µgL^{-1} + BPA 25.0 µM for each gene were calculated using Student's *t*-test (*** $p < 0.001$; **** $p < 0.0001$); ns = not significant.

3.3. Lipid Content

Nile red staining at 72 h of exposure revealed differences in lipid accumulation among the experimental groups. For this experiment, only the 20.0 μgL^{-1} HTs concentration was chosen also for the co-exposure. Larvae exposed to BPA 25.0 µM showed a strong increase in lipid fluorescence signal (Figure 5B) compared to the control group (Figure 5A). This effect was significantly attenuated in embryos co-exposed to BPA 25.0 µM and HTs 20.0 μgL^{-1} (Figure 5C). No significant differences were observed in the groups exposed to HTs 20.0 μgL^{-1} alone (Figure 5D) and DMSO 0.01% (Figure 5E). These results were confirmed by a semi-quantitative analysis of fluorescence intensity reported in the graph (Figure 5F).

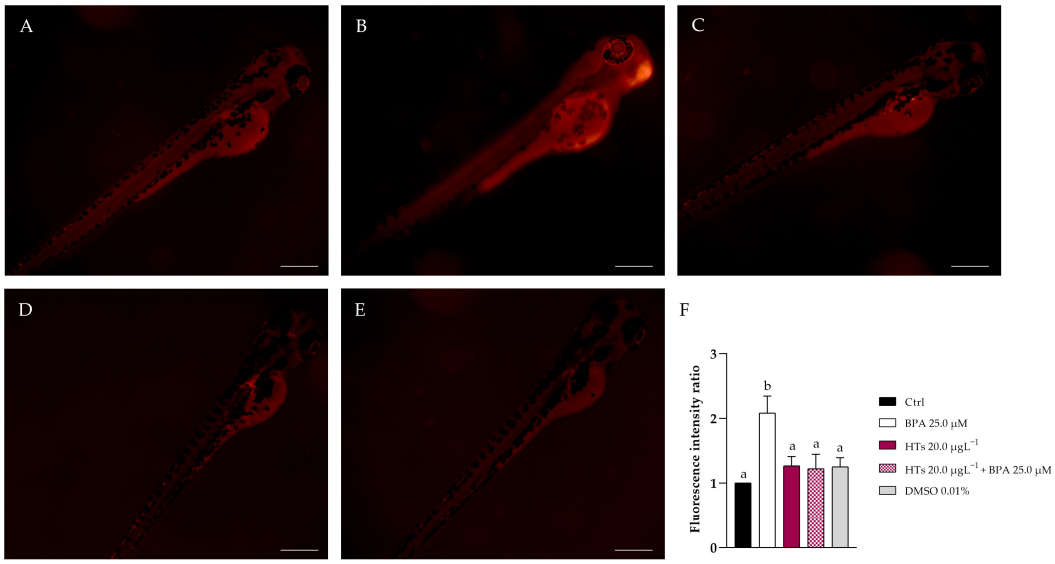

Figure 5. Nile red staining at 72 h of exposure (n = 3). (**A**) Ctrl; (**B**) BPA 25.0 µM; (**C**) HTs 20.0 μgL^{-1} + BPA 25.0 µM; (**D**) HTs 20.0 μgL^{-1}; (**E**) DMSO 0.01%. Scale bar: 200 µm. (**F**) Semi-quantitative analysis of fluorescence intensity. One-way ANOVA followed by Tukey's test: different lowercase letters indicated significant differences between groups.

4. Discussion

In our previous study, we demonstrated the dual role of hydrolysable tannins. At high concentrations (5.0, 10.0, and 20.0 mgL^{-1}), they were toxic in zebrafish embryonic development, altering the hatching process, while at low concentrations (5.0, 10.0, and 20.0 μgL^{-1}), they were harmless [25]. For this reason, low concentrations were chosen to test their protective effects in the presence of a toxic insult.

The results of this work showed that only the concentration at 20.0 μgL^{-1} had a protective effect. This could be explained considering the data reported by La Pietra et al. [25], which demonstrated that the effectiveness of tannins depends on their bioavailability and metabolism. Specifically, HTs are metabolized into pyrogallol (PY) and gallic acid (GA), but at low concentrations (5.0 and 10.0 μgL^{-1}), probably the absorption and production of these metabolites could be insufficient to produce a significant protective effect. In fact, the absorption and metabolism of these derivates is rapid, especially for gallic acid [33]. The lower concentrations (5.0 and 10.0 μgL^{-1}) lead to insufficient bioavailability of the active metabolites, which are unable to counteract the effects.

Being an endocrine disruptor, it is well-known that BPA causes delays in hatching, phenotypic alterations, and changes in heart rate [34,35]. The delay in hatching caused by BPA could be due to its binding to the thyroid hormone receptor. This bind interferes with the thyroid hormones essential for growth and development [35]. Other explanations

could be significant oxidative stress by increasing reactive oxygen species (ROS), which compromise overall embryonic development [36], or an inhibition of the Wnt/β-catenin signaling pathway, crucial for growth [37]. HTs at the maximum concentration tested (20.0 μgL^{-1}) were able to reduce the delay in hatching caused by BPA. This effect could be explained by several hypotheses. Firstly, tannins can improve thyroid function by counteracting the action of BPA, as demonstrated by [38] in which the action of a polyphenol (sesamin) improved thyroid regulation following the action of a toxic agent in zebrafish. Secondly, tannins can counteract the oxidative stress caused by BPA through their ion chelation action [39,40]. In particular, polyphenols sequester free iron (Fe^{2+}) by blocking the Fenton reaction and thereby preventing the formation of ROS [41]. Tertiarily, polyphenols activate Wnt/β-catenin signaling, restoring this pathway and consequently promoting embryo hatching [42].

As for the bradycardia observed in the group exposed to BPA, this could be due to its action as a xenoestrogen, thus binding to the ERs present in the heart [43]. In addition, BPA may interfere with calcium homeostasis, blocking ion channels during contraction and relaxation of cardiac tissue [44]. The alteration of the normal heartbeat by BPA also alters blood flow, causing fluid accumulation and the formation of pericardial edema. The formation of edema may also be due to an increase in ROS, which alters cell permeability, causing fluid to leak into the pericardial cavity [35,45,46].

It has already been demonstrated that tannins protect against cardiotoxicity induced by BPA [18]. In fact, they act as competitive antagonists by binding to the same ER sites as BPA, effectively reducing the negative influence of the endocrine disruptor on cardiac function [47]. In addition, polyphenols may counteract the action of BPA on calcium ion channels by activating sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase pumps (SERCA) and regulating cardiac function [48]. Furthermore, as already mentioned, polyphenols can counteract the formation of ROS, thus also preventing the formation of cardiac morphological alterations. The action of HTs against the toxic effects of BPA could be due to its metabolites. GA and PY possess hydrophenolic (-OH) groups that function as electron donors. These groups bind free radicals, stabilizing them and preventing cellular damage [49]. Instead, PY has been observed to inhibit the activity of enzymes that catalyze the formation of ROS, such as xanthine oxidase. This reduces the production of free radicals in the context of oxidative damage [50].

Molecular analysis confirmed what was observed phenotypically. The genes involved in hatching (*cd63*, *zhe1*, and *klf4*) and in heart development (*hand2*, *sox9b*, and *gata4*) were down-regulated in the group exposed to BPA alone, as previously demonstrated [51–54] and in line with our phenotypic data. In fact, BPA can increase or decrease the methylation of specific gene promoters, directly altering DNA methyl transferase or interfering with the hormonal pathways that regulate DNA methyl transferase, affecting transcription [55,56]. During co-exposure with HTs at 20.0 μgL^{-1}, an improvement in gene expression was observed compared to the group exposed to BPA alone. Polyphenols have been shown to improve gene expression by acting on epigenetic mechanisms. In fact, they can interact with proteins involved in methylation by opening and closing chromatin [57]. The action of polyphenols in gene expression has already been demonstrated. The epigallocatechin gallate was able to neutralize the alteration of *hand2* in presence of BPA [18] while resveratrol was able to improve the gene expression of *gata4* or *sox9b* in presence of trichloroethylene in zebrafish embryos [58].

Bisphenol A, in addition to being an endocrine disruptor, has also been defined as obesogenic since it alters lipid metabolism [59]. Exposure to BPA has been shown to cause an increase in glycerophospholipids and sphingolipids, which are also linked to cardiovascular disease and metabolic disorders. So, changes in lipid metabolism can cause

alterations in development [60]. The data from Nile red staining showed an increase in lipids in the group exposed to BPA alone, consistent with data on slowed embryonic development. In the co-exposure group, HTs normalized the lipid content alteration caused by BPA, as the control group. These results are according to Meguro et al. [61] who showed how coffee polyphenols decrease cholesterol accumulation, down-regulating cholesterol and lipoprotein synthesis and up-regulating the synthesis of bile acids. Pardal et al. [62] demonstrated the ability of resveratrol to reduce fat in zebrafish. Therefore, hydrolysable tannins, like all polyphenols in general, can regulate lipid metabolism [63,64]. In fact, GA interacts with free radicals and reduces the oxidation of low-density lipoproteins, a key factor in several diseases such as cardiovascular diseases [65].

5. Conclusions

This study investigated the potential protective role of HTs at low concentrations. The results showed that HTs were able to mitigate the toxic effects of bisphenol A during the early stages of zebrafish development by regulating gene expression levels and normalizing lipid content. These findings highlight the protective and anti-inflammatory properties of HTs and suggest that they could potentially exert protective effects in response to other environmental stressors to which organisms are increasingly exposed in daily life. Future studies will be necessary to determine whether similar protective mechanisms occur under different contaminant exposures.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments12120488/s1. Table S1: Primer sequences used for qRT-PCR. Table S2: Statistical analysis. Table S3: Statistical analysis of gene expression. BPA 25.0 μM against HTs 20.0 μgL^{-1} + BPA 25.0 μM. Figure S1: Larve exposed to HTs 20.0 μgL^{-1} (G, I) and to DMSO (H, J) at 72 h of exposure. Scale bar: 450 μm. References [66–70] are cited in the Supplementary Materials.

Author Contributions: Conceptualization, A.L.P. and I.F.; methodology, A.L.P. and I.F.; software, A.L.P. and T.M.; validation, I.F.; formal analysis, A.L.P., T.M. and I.F.; investigation, A.L.P. and T.M.; resources, I.F.; data curation, A.L.P.; writing—original draft preparation, A.L.P. and T.M.; writing—review and editing, A.L.P., T.M. and I.F.; visualization, I.F.; supervision, I.F.; project administration, I.F.; funding acquisition, I.F. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: According to National Italian (D.lgs 26/2014), European (2010/63/EU) and FELASA guidelines regarding the welfare of animals used for research purposes, no ethical authorization was needed for these experiments because they were performed in larvae before 5 days post-fertilization.

Data Availability Statement: Data are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

HTs	Hydrolysable tannins
BPA	Bisphenol A
POPs	Persistent organic pollutants

qRT-PCR	Quantitative real time PCR
NR	Nile red
PY	Pyrogallol
GA	Gallic acid

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