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Research Article

Enzymatic, Immunological, Biochemical, and Antioxidative Responses of the Rainbow Trout to Dietary p-Coumaric Acid upon Exposure to Ronstar Herbicide

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The use of herbicides has risen considerably in order to increase agricultural production, and Ronstar® is one of the popular organochlorine herbicides with oxadiazon as its active component. This herbicide has a wide range of effects on fish, including physiological, genetic, neurological, and hemato-immunological impacts. In the current study, p-Coumaric acid (P-CA) was used as a feed additive for its potential benefits in rainbow trout (*Oncorhynchus mykiss*) exposed to Ronstar using hematological, immunological, and biochemical analyses. The fish (16.02 ± 0.27 g) was divided into eight treatments as follows: C (negative control), P1, P2, and P3 (0.5, 1, and 1.5 g P-CA/kg, respectively, with no toxin), R1, P1R1, P2R1, and P3R1 (12.5% Ronstar toxin with 0, 0.5, 1, and 1.5 g P-CA/kg, respectively), each with a replicate of three. According to the two-way ANOVA test ($P < 0.05$), the overall trend of changes were growth, antioxidant enzyme activity (superoxide dismutase, catalase, glutathione peroxidase), and malondialdehyde level, humoral immune system (total immunoglobulin levels (total Ig)), lysozyme, complement 3 (C3), complement 4 (C4), ACH_{50}, serum nitroblue tetrazolium, and myeloperoxid. The only parameters that were unaffected by P-CA were globulin, albumin, and cortisol; however, Ronstar had negative effects on all three of these measures as well. It was found that the positive effects of dietary P-CA may ameliorate the negative effects of Ronstar in rainbow trout in a dose-dependent manner, with the best performance for the treatment P-CA + Ronstar. However, it is crucial to evaluate the mitigating effects of dietary P-CA against Ronstar on some vital organs of rainbow trout, especially the liver, at histological levels in future studies.

1. Introduction

Over the past 25 years, there has been a substantial global surge in the production and utilization of pesticides, driven by the objective of significantly augmenting food production [1]. The utilization of pesticides has undeniably emerged as a pivotal element within modern agricultural practices in recent times [2]. Herbicides are commonly utilized in terrestrial agricultural practices, accounting for approximately half of all pesticides employed in global agriculture. However, these substances have the potential to enter water resources either through drainage systems or intentional human activities. [3, 4]. The current state of the aquatic ecosystem is increasingly burdened by the indiscriminate and expanding use of herbicides, which presents a significant peril to the fragile water quality. The aforementioned process induces changes in the chemical composition of the water, subsequently influencing the organisms inhabiting the area [5]. Previous studies have indicated that herbicides can have adverse effects on fish, such as reduced reproduction, alterations in behavior, loss of biodiversity, and genetic, neurological, and hemato-immunological disorders [6–9].

The herbicide Ronstar®, which contains oxadiazon as its active ingredient, is extensively utilized in various applications.

The compound in question is classified as a category II organochloride herbicide, exhibiting a moderate level of toxicity [10]. According to the Bayer Material Safety Data Sheet, the pesticide formulation includes supplementary compounds in the form of surfactants (Regulation EC No, 1907/2006). The compounds encompassed within this group consist of cyclohexanone, ethoxylated polyaryl phenol, 2-methyl propane-1-ol, and calcium salts derived from C11 to C13 branched alkyl derivatives of benzene sulfonic acid [11]. Ronstar is commonly utilized for weed management in various agricultural settings, including rice, turf, soybean, and onion fields. According to the Washington State Department of Transportation, the average half-life of Ronstar® is approximately 60 days [12].

Herbal extracts have garnered significant attention due to their inherent origins and the multitude of advantageous effects [13–17]. Herbal supplements commonly possess anti-inflammatory and immunostimulatory properties that help fish to endure adverse environmental conditions [18–21]. Polyphenols are bioactive compounds of botanical origin that have been scientifically demonstrated to possess various biological functions and potential health benefits [22–24]. A multitude of studies have investigated the utilization of polyphenols as supplementary additives in the field of aquaculture within the previous decade [25]. Various categories of polyphenolic compounds, such as phenolic acids, flavonoids, stilbenes, and lignans, have been recognized to modulate the immune system and overall physiological functioning of fish [26, 27]. One example of a naturally occurring polyphenol possessing antioxidant properties is p-Coumaric acid (P-CA), which is a hydroxyl derivative derived from cinnamic acid [28]. The *in vitro* experiments conducted on P-CA [29, 30] have proven its antioxidant and radical scavenging activities. The application of P-CA inhibited the growth of bacteria [31]. Common carp (*Cyprinus carpio*) and rats have shown promise antioxidative, immunohumeral, antiseptic, and antibacterial benefits from P-CA, although there are not enough data on its usage as a food supplement in aquatic animals [32–34]. The primary deleterious effects of Ronstar on organisms are ascribed to its oxidative properties, rendering the organisms more susceptible to subsequent infections. Hence, the utilization of P-CA's antioxidant and antibacterial properties may present potential benefits in mitigating the Ronstar-related detrimental effects.

Rainbow trout is one of the most commercially important fish species in aquaculture. This assessment was carried out through the analysis of hematological, immunological, and biochemical parameters. It has been demonstrated that rainbow trout has a limited tolerance to environmental toxins, including herbicides, and that the immunological and biochemical components of its blood react rapidly to environmental toxins [35].

Consequently, the current study aimed to conduct a thorough evaluation of the potential advantageous effects of P-CA as a dietary supplement in rainbow trout (*Oncorhynchus mykiss*) exposed to Ronstar.

2. Material and Methods

2.1. P-CA Antioxidant Potentials

2.1.1. DPPH-Free Radical Scavenging Activity. To measure DPPH at 517 nm, 0.1 ml of the DPPH solution in ethyl alcohol was prepared and added (0.5 ml) to the p-coumaric solution (1.5 ml) in ethyl alcohol. The solution was vortexed and incubated for 30 min upon dark conditions [36]. Then, the absorbance of the solution was recorded at 517 nm. The percentage of DPPH was calculated as follows:

$$\text{Inhibition}\,(\%) = [(\text{Ac} - \text{Ap})/\text{Ac}] \times 100. \quad (1)$$

Ac, the absorbance for control; Ap, the absorbance for P-CA.

2.1.2. $ABTS^+$ Scavenging Activity. ABTS scavenging activity was assayed based on those previously described method [37]. $ABTS^+$ was generated upon the reaction of ABTS (7 M) in water and potassium persulfate (140 mM) and then incubated under dark for 30 min. Before use, $ABTS^+$ solution was diluted to 2.2 ± 0.05 mM using 0.1 M phosphate buffer. Then, $ABTS^+$ (1 ml) was added to P-CA (3 ml). After 30 min, the absorbance was read at 734 nm by a spectrophotometer and calculated as follows:

$$\text{ABTS radical scavenging activity}\,(\%) = [(\text{Ac} - \text{Ap})/\text{Ac}] \times 100. \quad (2)$$

Ac, the absorbance for control; Ap, the absorbance for P-CA.

2.1.3. Superoxide Radical Scavenging Activity (SRSA). In this method, 1 mM of nitro-blue tetrazolium, 1 ml of NADH solution, and 0.1 mM coumaric in ethyl alcohol were mixed. With adding 100 μM of phenazine methosulphatein 100 mM phosphate buffer, the reaction was activated, left for 6 min at 26°C, and the adsorption rate was read at 560 nm. The decrease in adsorption indicated an increase in the activity of superoxide ions. SRSA (%) was calculated as follows [38]:

$$\text{Inhibition}\,(\%) = [(\text{A0} - \text{A1})/\text{A0}] \times 100. \quad (3)$$

2.2. LC_{50} Measurements. LC_{50} of Ronstar was determined after estimating its lethal and acute concentrations on common carp. Then, the test doses were selected. To obtain the lethal range, the fish (16.02 ± 0.27 g, $n = 30$ in three repetitions) were exposed to experimental dietary levels of Ronstar (0.05, 0.10, 0.20, 0.40, 0.80, 1.60, 3.00, 6.00 mg/l) according to the standard method [39], with a control group (without Ronstar) after the adaptation period. The losses were recorded at 24, 48, 72, and 96 hr postexposure. Probit analysis was used to estimate LC_{30}, LC_{50}, LC_{70}, and LC_{90} values at 24, 48, 72, and 96 hr, respectively.

Table 1: Feedstuffs and compositions of the basal diet [40].

Ingredients	g/kg	Proximate composition	In dry basis (%)
Fish meal[a]	310	Crude protein	425
Soybean meal[b]	200	Crude lipid	163
Wheat meal	166	Crud ash	79.5
Poultry byproduct[c]	130	Moisture	88.2
Wheat gluten[d]	100		
Phytase[e]	8	—	—
Fish oil	40	—	—
Lysine[f]	7	—	—
Soybean oil	30	—	—
Methionine[f]	4	—	—
Mineral mix[g]	2.5	—	—
Vitamin mix[g]	2.5	—	—
Total	1,000	—	—

[a]Peygir Co., Gorgan, Iran (crude protein 58.8%). [b]Soyabean Co., Gorgan, Iran (crude protein 45.2%). [c]Peygir Co., Gorgan, Iran (crude protein 51.0%). [d]Shahdineh Aran Co (crude protein 78.3%). [e]Golbid Co., Tehran, Iran. [f]Mad Tiour Co., Sanandaj, Iran. [g]The premix provided following amounts per kg of feed: A: 1,000 IU; D3: 5,000 IU; E: 20 mg; B5: 100 mg; B2: 20 mg; B6: 20 mg; B1: 20 mg; H: 1 mg; B9: 6 mg; B12: 1 mg; B4: 600 mg; C: 50 mg; Mg: 350 mg; Fe: 13 mg; Co: 2.5 mg; Cu: 3 mg; Zn: 60 mg; Se: 0.3 mg; I: 1.5 mg; Mn: 10 mg). Chinechin Co., Tehran, Iran.

2.3. Fish and Treatments. In total, 650 rainbow trout juveniles (mean weight of 11.10 ± 0.20 g) provided from an owned farm located in the Fars province were considered for the experiment. The fish were adapted to laboratory conditions for a duration of 2 weeks, with a temperature range of 14–15°C. The fish were fed a basal diet (Table 1). After the adaptation period, the fish 16.02 ± 0.27 g (mean ± SE) were divided into eight treatments with three replications (600 fish were randomly distributed in 18 tanks with a density of 25 fish per replication). The experimental treatments were treatment C, the negative control group with no toxin and supplement; treatments P1, P2, and P3 that received no toxin and the diet contained 0.5, 1, and 1.5 g P-CA/kg, respectively; treatments R1, P1R1, P2R1, and P3R1 that received 12.5% Ronstar (oxadiazon) toxin with a diet contained 0, 0.5, 1, and 1.5 g/kg of P-CA, respectively.

During the rearing period (30 days), 80% of the water was changed every 12 hr and the same rate of lost Ronstar solution was poured into the tanks with the exchange water [41]. The suspended particles were removed daily, tanks continuously areated, and the biomass in tanks was adjusted every 2 weeks by weighing the fish to estimate the growth rate. Physicochemical factors of water maintained at 16.14 ± 0.5°C, dissolved oxygen: 8.2 ± 0.20 mg/l, pH: 7.3–7.5, and nonionized ammonia 0.01 ± 0.005. Feeding was done three times a day (at 08:00, 13:00, and 19:00 hr) and based on apparent satiety [42].

2.4. Preparation of the Experimental Diets. The ingredients used in the basic diet in this study are presented in Table 1. The components of the diet were mixed and then turned into a paste with some water. The paste was converted into pellets by an industrial meat grinder and the pellet was kept at room temperature for 36 hr and then refrigerated. Different levels of P-CA were added at the pasting step. Dose selection in this study was according to the positive results of previous researches [32, 33].

2.5. Sampling. At the end of the experimental period (Day 30), the feeding of fish was stopped for 24 hr, and the fish was anesthetized using cloves oil (100 mg/l; [43]) and growth indices were calculated as follows:

$$\text{Weight gain (WG, g)} = \text{Final weight} - \text{Initial weight}, \tag{4}$$

$$\text{Feed conversion ratio (FCR)} = \text{Feed intake}/(\text{FW} - \text{IW}), \tag{5}$$

$$\text{Specific growth rate (SGR, \%/day)} = 100 \times [(\ln \text{FW} - \ln \text{IW})/\text{day}], \tag{6}$$

$$\text{Survival rate (SR, \%)} = (\text{Nf}/\text{Ni}) \times 100. \tag{7}$$

To assess serum immune components, fish ($n = 3$) were anesthetized from each replicate using clove oil (100 mg/l; [43]). Blood samples were collected via the caudal vasculature using a 2 ml syringe. Serum samples were centrifuged (3,000x *g* for 10 min at 4°C) and kept at −70°C until the subsequent immunological assessments [44]. To collect mucus samples, the randomly selected fish ($n = 3$) were of each tank poured into saline solution (50 mM) in polyethylene bags saline, and after 5 min, the mucus accumulated in the bags was centrifuged (27,500x *g* for 5 min at 4°C), and the collected supernatant was kept at −80°C.

2.6. The Activity of Digestive Enzymes. Digestive enzyme activities were estimated after sacrificing fish ($n = 3$) using a high dosage of clove oil [45] and dissection of the fish. The intestine tissue samples were removed, emptied, washed by distilled water, mechanically homogenized in Tris buffer (50 mM), centrifuged (6,500x *g* for 10 min, 4°C), and the supernatant was stored for later analysis at −80°C. Amylase activity was measured upon action of the enzyme on 2% starch as substrate in phosphate buffer (0.1 M) [46]. Lipase enzyme activity was measured at 405 nm upon action of the enzyme on p-nitrophenyl myristate as substrate [47]. Protease activity was assayed at 440 nm upon action of the enzyme on azo-casein as substrate, according to García-Carreño [48]. Assessments of liver enzymes and biochemical parameters

Liver enzymes, including aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and alanine aminotransferase (ALT), were determined by commercial kits (Pars Azmun, Co., Tehran, Iran), according to the manufacturer's instructions. Total protein (TP) was measured by Bradford [49] method. Albumin was measured by the colorimetric method described by Nicholson [50] at 620 nm. Globulin concentrations were measured by subtraction of TP and albumin content [49, 50].

2.7. Measurements of Glucose and Cortisol. Cortisol was measured using a commercial kit (IBL Company, Germany) based on an ELISA method. Glucose was also measured by Pars Azmun commercial kit [51].

2.8. Immune Components. Lysozyme activity in serum and mucus was assessed based on the turbidity method using *Micrococcus lysodeikticus* (0.2 mg/ml) as the target in a 0.05 M sodium phosphate buffer (pH 6.2) [52]. Total serum and mucus total immunoglobulin (total Ig) were measured by measuring the protein content of the samples before and after reaction with polyethylene glycol. The complement components (C3 and C4) in serum were estimated using an ELISA reader using assay kit (Pars Azmun Company, Iran). Serum complement pathway activity (ACH_{50}) was calculated according to Yano [53] using sheep erythrocytes in vernal tissue, including EGTA and manganese as targets. Different concentrations (0.312%, 0.625%, 1.25%, 2.5%, 5%, and 10%) of the serum samples were prepared, and the serum samples (25 μl) were mixed with buffer (125 ml) and blood cells (50 μl). After 2 hr of incubation at room temperature, the mixture was centrifuged and the absorbance was recorded at 412 nm.

Myeloperoxidase activity of the serum (MPO) was measured at 450 nm with the addition of 90 μl of Hanks Balanced Salt Solution (HBSS) without Ca^+ and Mg^+ (Sigma-Aldrich) in 10 μl of serum in 96-well cell culture plates. Then, 3,3,5,5 tetramethylbenzidine hydrochloride (35 μl) (Sigma-Aldrich) was added, and afterward, the reaction was stopped by 0.5 M sulfuric acid [54]. Serum nitroblue tetrazolium (NBT) was measured based on the method of a previous study [55]. In summary, 100 μl of heparinized blood was mixed with 100 μl of NBT (0.2%) and incubated at 26°C for 20 min. The suspension (50 μl) was added to N, N-dimethylformamide (1 ml), and centrifuged at 3,600 for 4 min, and the rate of adsorption in the supernatant was assayed at 540 nm.

Mucus protease activity was assessed using the method of García-Carreño [48]. About 100 μl of the sample was mixed with 0.7% azocasein-mixed ammonium bicarbonate (100 μl) buffer and then incubated at 25°C for 24 hr. The reaction was stopped in the presence of trichloroacetic acid, and the supernatant was collected after centrifugation (15,000x *g* for 5 min). Then, 0.5 N hydroxide was added to the supernatant, and the adsorption rate was recorded at 450 nm. Peroxidase activity in mucus was assessed using Hanks Balanced Salt Solution (HBSS) at a wavelength of 450 nm [54]. Alkaline phosphatase (ALP) activity in mucus was measured using Pars Azmoun commercial kit using the manufacturer's instructions.

2.9. Measurement of Antioxidant Enzyme Activity. Superoxide dismutase (SOD) activity was estimated by a commercial kit (Zellbio, Hamburg, Germany) upon the reduction rate of cytochrome *C* [56]. Catalase (CAT) activity was assayed upon the decomposition rate of hydrogen peroxidase, according to Goth [57]. Glutathione peroxidase (GPx) activity was determined upon measuring the converting rate of glutathione to glutathione disulfide using a commercial kit (Zellbio, Hamburg, Germany) according to Hoseini et al. [56]. Malondialdehyde (MDA) was estimated by a commercial kit upon a thiobarbituric acid reaction at 95°C (Zellbio, Hamburg, Germany).

Table 2: Lethal concentrations (LC_{1-90}) of Ronstar depending on time (24–96 hr) for rainbow trout (mean ± SE).

Point	Concentration (mg/l) (95% of confidence limits)			
	24 hr	48 hr	72 hr	96 hr
LC_{30}	2.83 ± 0.13	2.13 ± 0.11	1.56 ± 0.09	0.49 ± 0.2
LC_{50}	**3.42 ± 0.13**	**2.74 ± 0.11**	**2.27 ± 0.09**	**0.82 ± 0.2**
LC_{70}	4.01 ± 0.13	3.35 ± 0.11	2.99 ± 0.09	1.15 ± 0.2
LC_{90}	4.87 ± 0.13	4.22 ± 0.11	4.02 ± 0.09	1.63 ± 0.2

The bold values signify the sub-lethal concentrations of the pesticide that are determined based on LC_{50}.

Table 3: Evaluation of the antioxidant power of P-CA (p-Coumaric acid) using different methods.

DPPH inhibition (%)	51.24 ± 1.04
ABTS radical scavenging activity (%)	48.25 ± 1.51
Superoxide anion radical (%) scavenging activity	35.5 ± 2.05

2.10. Statistical Analysis. The experiment was done in a completely randomized design with eight treatments in three replications. Data analysis was carried out using SPSS software version 20. The data normality and the homogeneity of variances were confirmed using the Shapiro–Wilk test and Levene tests. Two-way ANOVA and Tukey multiple domain tests were used for data analysis. The results were presented based on the means and standard errors with a significance level of 0.05.

3. Results

3.1. LC_{50} Measurements. LC_{50} of Ronstar in 24–96 hr was calculated for rainbow trout (Table 2).

3.2. Antioxidant Activity of P-CA. In order to confirm the antioxidant activity of P-CA, DPPH-free radical scavenging activity, ABTS radical scavenging activity (%), and superoxide anion radical (%) scavenging activity were measured (Table 3).

3.3. Effect of P-CA and Ronstar on Growth Parameters. The effect of P-CA on growth parameters in rainbow trout exposed to Ronstar is presented in Table 4. There was no significant difference between treatments in initial weight and survival rate. The observed differences for the final weight and gained weight indices were consistent with each other, and in both indices, treatment R1: 12.5% Ronstar without P-CA supplementation had the lowest final and gained weight, and treatment P2: Ronstar free along with 0.1 g P-CA/kg had the highest significant level ($P<0.05$). However, no significant difference was observed between treatment P2 and other treatments except treatments R1 and P1R1: 12.5% Ronstar with a diet containing 0.5 g P-CA/kg. The lowest significant ($P<0.05$) SGR (%/d) was observed in treatment R1, and there was no significant difference in other treatments. The highest significant ($P<0.05$) FCR was

Table 4: Effect of P-CA (p-Coumaric acid) on growth parameters in rainbow trout exposed to Ronstar ($P<0.05$).

Different experimental diets							
P-CA	Ronstar®	IW (g)	FW (g)	WG (g)	SGR (%/day)	FCR	SR (%)
0	0	16.03 ± 0.26^{a}	34.16 ± 0.44^{b}	18.13 ± 0.44^{b}	2.53 ± 0.06^{a}	1.26 ± 0.06^{b}	96.00 ± 3.46^{a}
0.5	0	15.65 ± 0.07^{a}	35.83 ± 1.01^{ab}	20.18 ± 1.07^{ab}	2.78 ± 0.11^{a}	1.16 ± 0.05^{b}	95.00 ± 1.72^{a}
1	0	15.96 ± 0.14^{a}	40.16 ± 0.88^{a}	24.20 ± 0.73^{a}	3.07 ± 0.04^{a}	1.04 ± 0.04^{b}	97.00 ± 3.00^{a}
1.5	0	16.20 ± 0.15^{a}	38.66 ± 0.87^{ab}	22.46 ± 1.04^{ab}	2.89 ± 0.10^{a}	1.08 ± 0.02^{b}	95.00 ± 1.72^{a}
0	12.5	16.10 ± 0.10^{a}	28.93 ± 0.78^{c}	12.83 ± 0.68^{c}	1.95 ± 0.06^{c}	1.80 ± 0.09^{a}	92.00 ± 1.72^{a}
0.5	12.5	16.06 ± 0.12^{a}	34.12 ± 0.60^{b}	18.10 ± 0.55^{b}	2.51 ± 0.08^{a}	1.26 ± 0.04^{b}	94.00 ± 3.0^{a}
1	12.5	16.03 ± 0.13^{a}	37.66 ± 0.88^{ab}	21.63 ± 0.86^{ab}	2.84 ± 0.13^{a}	1.11 ± 0.04^{b}	95.00 ± 4.58^{a}
1.5	12.5	16.16 ± 0.16^{a}	37.65 ± 1.64^{ab}	21.50 ± 1.52^{ab}	2.81 ± 0.21^{a}	1.14 ± 0.14^{b}	93.00 ± 1.72^{a}
Two-way ANOVA (P-value)							
P-CA		0.240	0.001	0.002	0.051	0.003	0.576
Ronstar®		0.253	0.001	0.001	0.204	0.171	0.067
Ronstar® × P-CA		0.503	0.168	0.158	0.842	0.452	0.824

The differences between the means are indicated as different superscript letters.

observed in treatment R1, and there was no significant difference in the other treatments.

In the FW and WG indices, the separate effects of Ronstar and P-CA were significant ($P<0.05$), but in other growth indices, the separate effects of these two variables were not significant except for the FCR index, in which only P-CA was significant ($P<0.05$) and Ronstar had no significant effect. Also, the effect of the interaction of these two variables was not significant in all growth indices.

3.4. Effect of P-CA and Ronstar on the Activity of Digestive Enzymes. The lowest significant ($P<0.05$) lipase activity was in treatment R1: 12.5% Ronstar without P-CA supplementation compared to other treatments. In general, there was no significant difference between treatments that received both 12.5% Ronstar and P-CA (P1R1, P2R1, and P3R1), P1: Ronstar free along with 0.5 g P-CA/kg, and the control group. The highest significant ($P<0.05$) lipase activity was observed in P2: Ronstar free along with 0.1 g P-CA/kg, which was not significantly different from P3: Ronstar free along with 1.5 g P-CA/kg (Figure 1(a)).

The highest significant activity of protease was observed in treatment P2: Ronstar free along with 0.1 g P-CA/kg, and the lowest activity was seen in the treatment R1: 12.5% Ronstar without P-CA supplementation ($P<0.05$). Except for these two treatments, no significant differences were found in other treatments in comparison with the control group. Also, there was no significant difference between treatment P2: Ronstar free along with 0.1 g P-CA/kg with maximum protease activity and treatments P1: Ronstar free along with 0.5 g P-CA/kg and P2R1: 12.5% Ronstar with a diet containing 1 g P-CA/kg (Figure 1(b)).

The treatments receiving 12.5% Ronstar and P-CA (P1R1, P2R1, and P3R1) and the control group showed no significant differences in amylase activity ($P>0.05$). The highest significant ($P<0.05$) amylase activity was observed in P2: Ronstar free along with 0.1 g P-CA/kg compared to the control group, which was not significantly different from P1 and P3 treatments (Ronstar free along with 0.5 and 1.5 g P-CA/kg, respectively). The lowest significant ($P<0.05$) amylase activity was observed in treatment R1: 12.5% Ronstar without P-CA supplementation compared to other treatments (Figure 1(c)).

The separate effect of Ronstar and P-CA was significant ($P<0.05$) for lipase, amylase, and protease activity. The interaction effect between Ronstar and P-CA was not significant for lipase and amylase activity, but their interactions were significant ($P<0.05$) for protease activity.

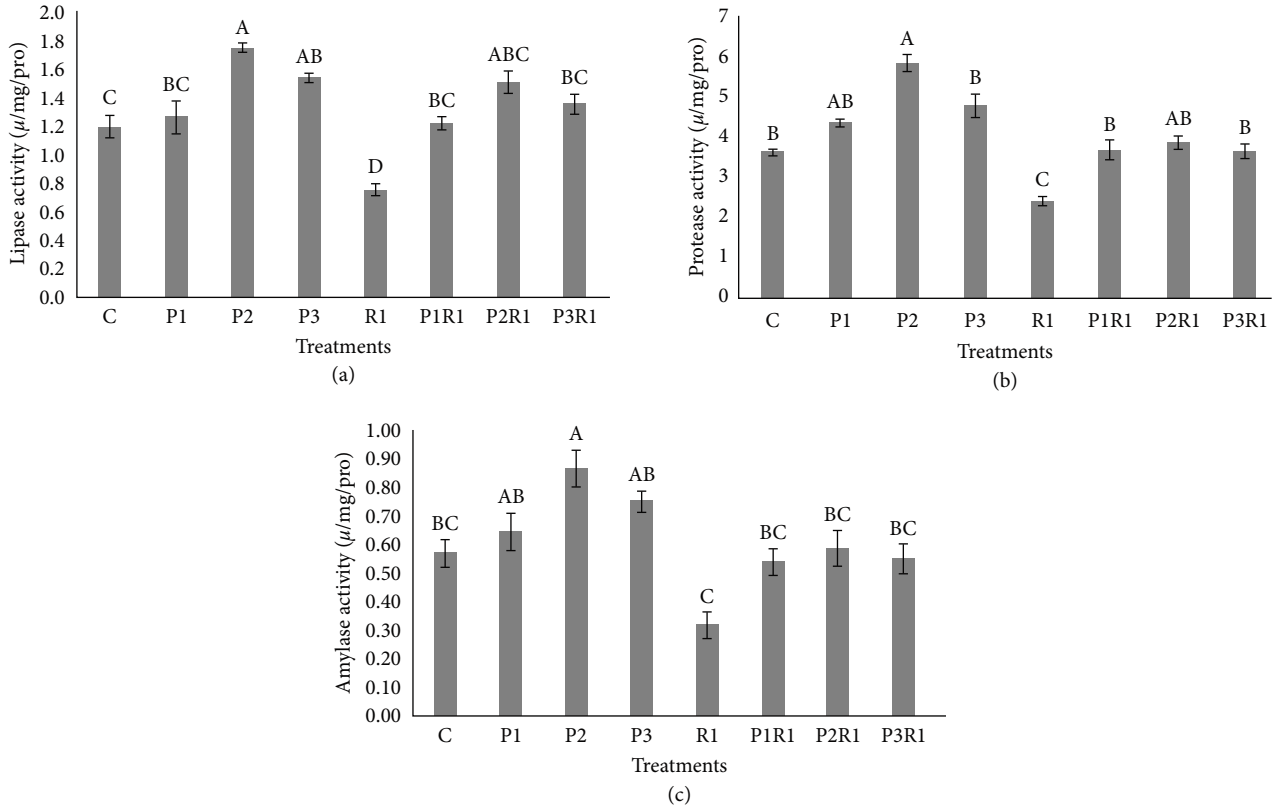
3.5. Effect of P-CA and Ronstar on Biochemical Parameters of Blood. The effect of P-CA and Ronstar on biochemical parameters of blood, including cortisol, glucose, TP, albumin, and globulin, is presented in Table 5.

All treatments that received P-CA with or without Ronstar exposure had significantly ($P<0.05$) lower cortisol levels compared to the control group, and there was no significant difference between them. R1: 12.5% Ronstar without P-CA was the only treatment that showed no significant difference in comparison with the control group. The highest significant 12.5% Ronstar without P-CA glucose levels was in R1 treatment, and the lowest was observed in treatments with 1 g P-CA/kg with or without Ronstar exposure (P2 and P2R1). The values of TP, globulin, and albumin had no significant differences between the groups, except for treatment R1, with significantly ($P<0.05$) the lowest TP level.

The effect of P-CA was significant ($P<0.05$) in all assayed biochemical parameters, except for albumin. The separate effect of Ronstar was also significant ($P<0.05$) for assayed biochemical parameters, except for albumin and globulin. The interaction effect of these two variables was significant ($P<0.05$) for glucose, cortisol, and TP.

3.6. Effect of P-CA and Ronstar on Liver Enzymes. Effects of P-CA and Ronstar on liver enzymes, including LDH, ALP, AST, and ALT, are presented in Figure 2.

No significant difference was found for LDH levels between all experimental treatments, except for R1: 12.5% Ronstar without P-CA with the lowest significant ($P<0.05$) level. The highest significant ($P<0.05$) LDH level compared to



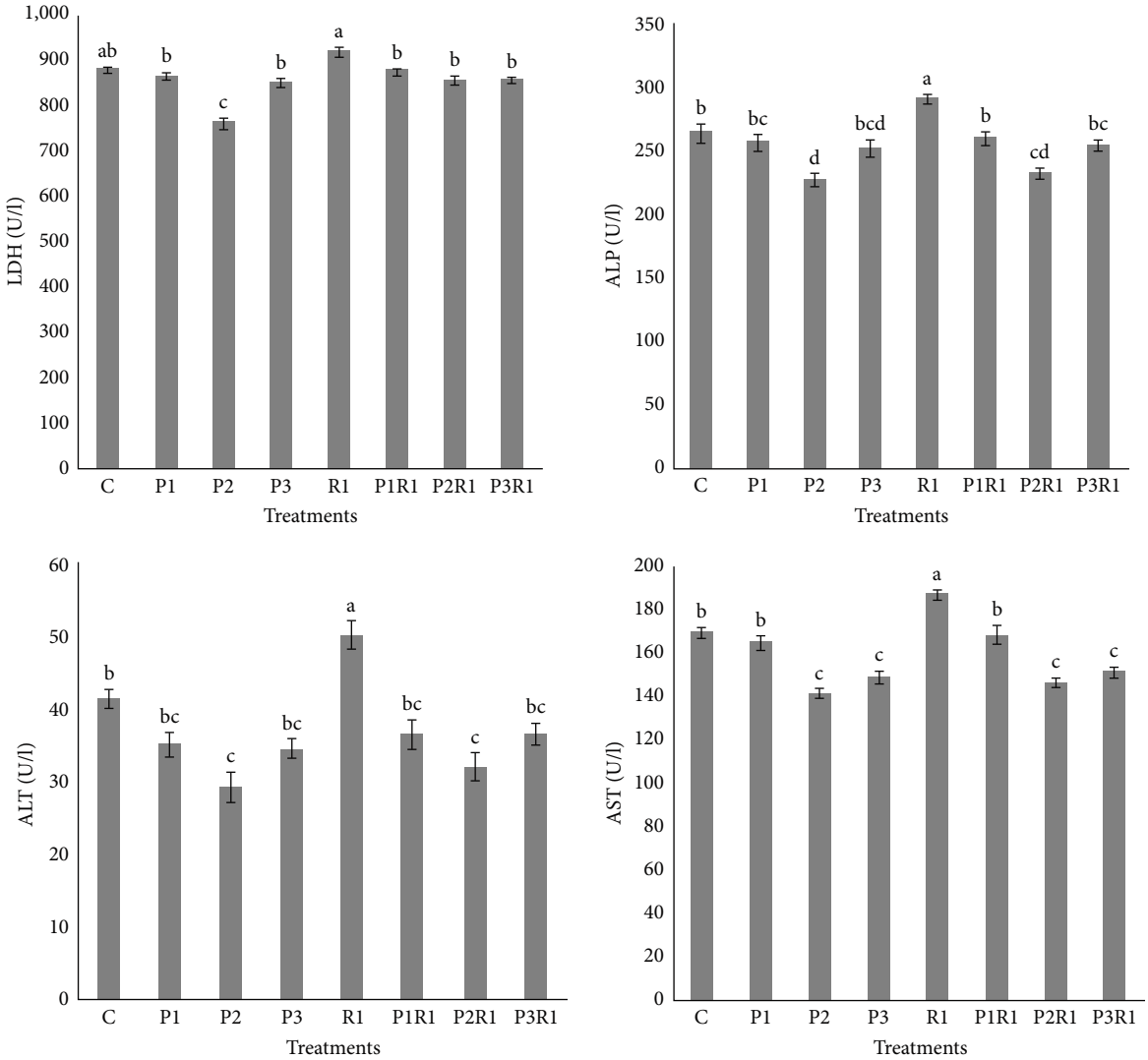
	Two-way ANOVA (*P*-value)		
	Protease (μ/mg/pro)	Lipase (μ/mg/pro)	Amylase (μ/mg/pro)
p-CA	0.001	0.001	0.001
Ronstar^{R}	0.001	0.002	0.001
Ronstar^{R}× p-CA	0.020	0.050	0.410

Figure 1: Effect of P-CA (p-Coumaric acid) on the activity of digestive enzymes in rainbow trout exposed to Ronstar ($P<0.05$). C: negative control, P1: 0.5 g P-CA/kg, P2: 1 g P-CA/kg, P3: 1.5 g P-CA/kg; R1: 12.5% Ronstar toxin, P1R1: 12.5% Ronstar toxin + 0.5 g P-CA/kg, P2R1: 12.5% Ronstar toxin + 1 P-CA/kg, P3R1: 12.5% Ronstar toxin + 1.5 P-CA/kg. Two-way ANOVA of the effect of P-CA (p-Coumaric acid) and Ronstar on the activity of digestive enzymes in rainbow trout ($P<0.05$).

Table 5: Effect of P-CA (p-Coumaric acid) on biochemical parameters in rainbow trout exposed to Ronstar ($P<0.05$).

Different experimental diets						
P-CA	Ronstar	Cortisol (ng/ml)	Glucose (mg/dl)	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)
0	0	152.83 ± 1.76^{ab}	84.88 ± 1.09^{b}	3.63 ± 0.12^{a}	2.10 ± 0.15^{a}	1.53 ± 0.08^{ab}
0.5	0	137.66 ± 1.45^{bc}	80.86 ± 1.04^{bc}	3.24 ± 0.08^{a}	2.06 ± 0.04^{ab}	1.18 ± 0.06^{bc}
1	0	128.51 ± 2.36^{c}	74.16 ± 1.48^{d}	3.90 ± 0.20^{a}	2.33 ± 0.17^{a}	1.60 ± 0.05^{a}
1.5	0	129.33 ± 3.54^{c}	79.50 ± 1.32^{bcd}	3.36 ± 0.4^{1a}	1.94 ± 0.22^{ab}	1.40 ± 0.05^{abc}
0	12.5	166.00 ± 3.47^{a}	97.10 ± 1.24^{a}	2.46 ± 0.12^{b}	1.40 ± 0.15^{b}	1.06 ± 0.04^{c}
0.5	12.5	140.16 ± 4.20^{bc}	81.83 ± 0.92^{bc}	3.26 ± 0.10^{a}	1.83 ± 0.12^{ab}	1.43 ± 0.05^{abc}
1	12.5	133.00 ± 3.21^{c}	78.36 ± 1.01^{cd}	3.56 ± 0.09^{a}	2.00 ± 0.05^{ab}	1.50 ± 0.10^{ab}
1.5	12.5	134.60 ± 3.81^{c}	80.20 ± 1.47^{bc}	3.40 ± 0.11^{a}	1.96 ± 0.12^{ab}	1.44 ± 0.14^{abc}
Two-way ANOVA (*P*-value)						
P-CA		0.001	0.001	0.003	0.080	0.022
Ronstar®		0.011	0.002	0.002	0.007	0.241
Ronstar® × P-CA		0.367	0.001	0.003	0.134	0.006

The differences between the means are indicated as different superscript letters.



	Two-way ANOVA (*P*-value)			
	ALT (U/l)	AST (U/l)	ALP (U/l)	LDH (U/l)
p-CA	0.007	0.002	0.019	0.002
Ronstar^{R}	0.001	0.001	0.010	0.001
Ronstar^{R}× p-CA	0.178	0.061	0.093	0.001

Figure 2: Effect of P-CA (p-Coumaric acid) on liver enzymes in rainbow trout exposed to Ronstar ($P<0.05$). C: negative control, P1: 0.5 g P-CA/kg, P2: 1 g P-CA/kg, P3: 1.5 g P-CA/kg; R1: 12.5% Ronstar toxin, P1R1: 12.5% Ronstar toxin + 0.5 g P-CA/kg, P2R1: 12.5% Ronstar toxin + 1 P-CA/kg, P3R1: 12.5% Ronstar toxin + 1.5 P-CA/kg. Two-way ANOVA of the effect of P-CA (p-Coumaric acid) and Ronstar on liver enzymes in rainbow trout ($P<0.05$).

other treatments but not the control group was observed in P2: Ronstar free treatments with 1 g P-CA/kg treatment.

The only treatments with a significant difference in ALP and ALT activity levels with the control group were R1 (the highest) and treatments that received 1 g P-CA/kg with or without exposure to the Ronstar (P2 and P2R1) with the lowest ALP and ALT activity levels.

Except for the treatments with 0.5 g P-CA/kg with or without exposure to Ronstar (P1 and P1R1), all other treatments showed significant ($P<0.05$) differences in the liver enzymes with the control group, with a significant level in R1.

Ronstar and P-CA separate effects were significant ($P<0.05$) in all assessed liver enzymes, but the interaction effect of these two variables was only significant for LDH activity.

3.7. Effect of P-CA and Ronstar on Immune Responses in Serum. P-CA and Ronstar affected immune responses in the fish (Table 6). Regarding the serum lysozyme activity, the highest significant ($P<0.05$) levels were related to

Table 6: Effect of P-CA (p-Coumaric acid) on serum immune responses in rainbow trout exposed to Ronstar ($P<0.05$).

Different experimental diets								
P-CA	Ronstar®	LYZ (U/ml)	Ig (mg/ml)	C3 (g/dl)	C4 (g/dl)	ACH_{50} (U/ml)	NBT (540 nm)	MPO (450 nm)
0	0	23.28 ± 0.53^{b}	15.59 ± 0.67^{cd}	14.58 ± 0.34^{b}	7.33 ± 0.56^{bc}	105.33 ± 3.16^{a}	$0.52 \pm .04^{b}$	$1.34 \pm .05^{cde}$
0.5	0	25.67 ± 0.56^{ab}	18.57 ± 0.81^{bc}	16.10 ± 0.55^{ab}	8.13 ± 0.58^{bc}	109.16 ± 3.03^{a}	0.71 ± 0.03^{ab}	1.61 ± 0.05^{ab}
1	0	27.06 ± 0.78^{a}	22.31 ± 0.60^{a}	18.33 ± 0.60^{a}	13.28 ± 0.44^{a}	117.34 ± 2.60^{a}	0.90 ± 0.04^{a}	1.80 ± 0.06^{a}
1.5	0	24.86 ± 0.39^{ab}	21.16 ± 0.52^{ab}	16.50 ± 1.04^{ab}	12.70 ± 0.52^{a}	115.66 ± 2.33^{a}	0.84 ± 0.05^{a}	1.56 ± 0.04^{abc}
0	12.5	19.20 ± 0.69^{c}	13.53 ± 0.70^{d}	10.26 ± 0.75^{c}	5.30 ± 0.40^{c}	85.33 ± 3.52^{b}	0.3 ± 0.05^{c}	$1.12 \pm .07^{e}$
0.5	12.5	24.46 ± 0.66^{ab}	17.83 ± 0.72^{bc}	15.53 ± 0.43^{ab}	7.41 ± 0.55^{bc}	106.66 ± 4.34^{a}	0.61 ± 0.04^{b}	1.25 ± 0.06^{de}
1	12.5	24.60 ± 0.52^{a}	18.36 ± 0.69^{bc}	17.46 ± 0.86^{ab}	9.06 ± 0.92^{b}	108.11 ± 2.88^{a}	0.72 ± 0.03^{ab}	1.50 ± 0.04^{bcd}
1.5	12.5	23.90 ± 0.83^{b}	18.06 ± 0.80^{bc}	15.50 ± 0.88^{ab}	8.40 ± 0.61^{b}	106.66 ± 4.33^{a}	0.58 ± 0.02^{b}	1.31 ± 0.03^{cde}
Two-way ANOVA (P-value)								
P-CA		0.001	0.001	0.001	0.001	0.001	0.001	0.001
Ronstar®		0.001	0.001	0.001	0.004	0.001	0.001	0.001
Ronstar® × P-CA		0.092	0.163	0.021	0.061	0.112	0.319	0.591

The differences between the means are indicated as different superscript letters.

treatments P2: Ronstar free along with 1 g P-CA/kg and P2R1: 12.5% Ronstar with a diet containing 1 g P-CA/kg, although no significant difference was found between these two treatments and treatments P1: Ronstar free along with 0.5 g P-CA/kg, P3: Ronstar free along with 1.5 g P-CA/kg and P1R1: 12.5% Ronstar with a diet containing 0.5 g/kg of P-CA. Also, there was no significant difference between treatments P1: Ronstar free along with 0.5 g P-CA/kg, P3: Ronstar free along with 1.5 g P-CA/kg, P1R1: 12.5% Ronstar with a diet containing 0.5 g P-CA/kg and P3R1; 12.5% Ronstar with a diet containing 1.5 g P-CA/kg and the control group. The lowest significant difference ($P<0.05$) was seen in R1: 12.5% Ronstar without P-CA supplementation.

Total Ig levels of serum exhibited no difference between Ronstar treatments with P-CA supplementation and treatment P1: Ronstar free along with 0.5 g P-CA/kg. The highest significant ($P<0.05$) level of total Ig was seen in treatment P2: Ronstar free along with 1 g P-CA/kg, which was not significantly different from treatment P3: Ronstar free along with 1.5 g P-CA/kg. The lowest significant ($P<0.05$) level of total Ig was observed in R1: 12.5% Ronstar without P-CA supplementation, which was not significantly different from the control group.

The complement C3 in serum showed no significant difference between treatments P1: Ronstar free along with 0.5 g P-CA/kg, P3: Ronstar free along with 1.5 g P-CA/kg, P2R1: 12.5% Ronstar with a diet containing 1 g P-CA/kg and P3R1; 12.5% Ronstar with a diet containing 1.5 g P-CA/kg and the control group. The highest significant ($P<0.05$) level was in treatment P2: Ronstar free along with 1 g P-CA/kg and the lowest significant ($P<0.05$) level in treatment R1: 12.5% Ronstar without P-CA supplementation.

There was no significant difference between treatments P1: Ronstar free along with 0.5 g P-CA/kg, P2R1: 12.5% Ronstar with a diet containing 1 g P-CA/kg, and P3R1; 12.5% Ronstar with a diet containing 1.5 g P-CA/kg, and the control group in relation to serum complement component 4 (C4) levels. The highest significant ($P<0.05$) level was observed in treatments P2: Ronstar free along with 1 g P-CA/kg and P3: Ronstar free along with 1.5 g P-CA/kg and the lowest significant ($P<0.05$) level was observed in treatment R1: 12.5% Ronstar without P-CA supplementation, which was not different from treatments P1: Ronstar free along with 0.5 g P-CA/kg, P1R1: 12.5% Ronstar with a diet containing 0.5 g P-CA/kg and P2R1; 12.5% Ronstar with a diet containing 1 g P-CA/kg.

ACH_{50} activity had no significant differences in values between all groups, except for R1: 12.5% Ronstar without P-CA supplementation treatment.

Regarding NBT and MPO, only treatments P2: Ronstar free along with 1 g P-CA/kg, P3: Ronstar free along with 1.5 g P-CA/kg, and R1: 12.5% Ronstar without P-CA supplementation were significantly ($P<0.05$) different from control treatment, with the highest levels being related to treatments P2, P3, and the lowest level being related to treatment R1.

In all assessed immune response parameters in the serum, P-CA and Ronstar separately had a significant ($P<0.05$) effect, but there was no significant interactive effect for these two variables except for C3.

3.8. Effect of P-CA and Ronstar on Immune Responses in the Mucus. The effects of P-CA and Ronstar on immune responses, including lysozyme, peroxidase, ALP, total Ig, and protease in the mucus of the fish, are presented in Table 7. Regarding lysozyme and ALP levels in mucus, only treatments P2: Ronstar free along with 1 g P-CA/kg and R1: 12.5% Ronstar without P-CA supplementation treatment were significantly ($P<0.05$) different from the control treatment. The highest significant ($P<0.05$) level was observed in treatment P2, while no significant difference was observed between this treatment and other treatments, except for treatment R1. The lowest significant ($P<0.05$) level was related to treatment R1.

In peroxidase activity, only treatments P2: Ronstar free along with 1 g P-CA/kg and R1: 12.5% Ronstar without P-CA supplementation treatment were significantly ($P<0.05$) different from the control treatment. There was no significant difference between R1 and P3: Ronstar free along with 1.5 g P-CA/kg. Besides, no significant difference was found between P2 and other treatments, except for R1 and the control treatment. P2 treatment was the only treatment with a significant

Table 7: Effect of P-CA (p-Coumaric acid) on mucosal immune responses in rainbow trout exposed to Ronstar ($P<0.05$).

Different experimental diets						
P-CA	Ronstar®	LYZ (U/ml)	ALP (U/l)	Peroxidase (U/ml)	Protease (%)	Ig (mg/ml)
0	0	10.04 ± 0.96^{bc}	29.51 ± 0.73^{bc}	8.33 ± 0.60^{b}	10.20 ± 0.70^{bc}	9.30 ± 0.51^{cd}
0.5	0	11.82 ± 0.73^{abc}	32.26 ± 0.81^{ab}	9.74 ± 0.55^{ab}	11.13 ± 0.44^{abc}	12.83 ± 0.44^{ab}
1	0	14.51 ± 0.82^{a}	34.50 ± 1.04^{a}	11.73 ± 0.38^{a}	13.63 ± 0.52^{a}	14.54 ± 0.60^{a}
1.5	0	11.18 ± 0.54^{abc}	32.13 ± 1.44^{ab}	7.33 ± 0.61^{b}	12.16 ± 0.72^{ab}	14.33 ± 0.43^{ab}
0	12.5	8.46 ± 0.57^{c}	26.23 ± 0.70^{c}	7.66 ± 0.62^{b}	8.70 ± 0.64^{c}	8.56 ± 0.66^{d}
0.5	12.5	11.63 ± 0.66^{abc}	30.73 ± 0.64^{ab}	9.56 ± 0.72^{ab}	10.06 ± 0.58^{bc}	11.63 ± 0.64^{bc}
1	12.5	13.46 ± 0.56^{ab}	31.73 ± 0.79^{ab}	9.76 ± 0.66^{ab}	11.16 ± 0.72^{abc}	12.63 ± 0.52^{ab}
1.5	12.5	11.36 ± 0.69^{abc}	30.26 ± 0.43^{abc}	9.30 ± 0.79^{ab}	10.23 ± 0.43^{bc}	12.60 ± 0.75^{ab}
Two-way ANOVA (P-value)						
p-CA		0.001	0.003	0.001	0.002	0.002
Ronstar®		0.156	0.001	0.633	0.001	0.004
Ronstar® × P-CA		0.469	0.738	0.044	0.698	0.747

The differences between the means are indicated as different superscript letters.

($P<0.05$) difference in protease percentage in comparison with the control group. Treatments P1R1: 12.5% Ronstar with a diet containing 0.5 g P-CA/kg, and R1 were the only treatments with no significant difference in comparison with control, but all other treatments had significantly ($P<0.05$) higher Ig levels.

The separate effect of P-CA was significant ($P<0.05$) in all assessed immunity response parameters in mucus, but the effect of Ronstar was not significant in peroxidase activity. On the other hand, peroxidase was the only parameter that Ronstar and P-CA had a significant ($P<0.05$) interactive effect on it.

3.9. Effect of P-CA and Ronstar on Antioxidative Activity. The effects of P-CA and Ronstar on antioxidative activity were assessed using SOD, CAT, GPx, and MDA (Figure 3).

All treatments showed significantly different SOD activity compared to the control treatment. The lowest significant ($P<0.05$) SOD activity was observed in R1: 12.5% Ronstar without P-CA, and the highest significant ($P<0.05$) activity was in Ronstar free treatments with 1 and 1.5 g P-CA/kg (P2 and P3).

Treatments that received 1 and 1.5 g P-CA/kg with or without exposure to Ronstar showed significantly ($P<0.05$) higher CAT activity, while R1 had the lowest significant ($P<0.05$) CAT activity. There was no significant difference between the rest of the treatments and the control group.

MDA activity of P2 (the lowest level), R1 (the highest level), and P2R1 had significant ($P<0.05$) differences with the control group. For GPx activity, the lowest significant activity was observed in R1: 12.5% Ronstar without P-CA, and the highest significant activity was in P2: Ronstar-free treatments with 1 g/kg P-CA. Treatments that received 0.5 g P-CA/kg with or without exposure to Ronstar (P1, P1R1) had no significant difference compared to the control group.

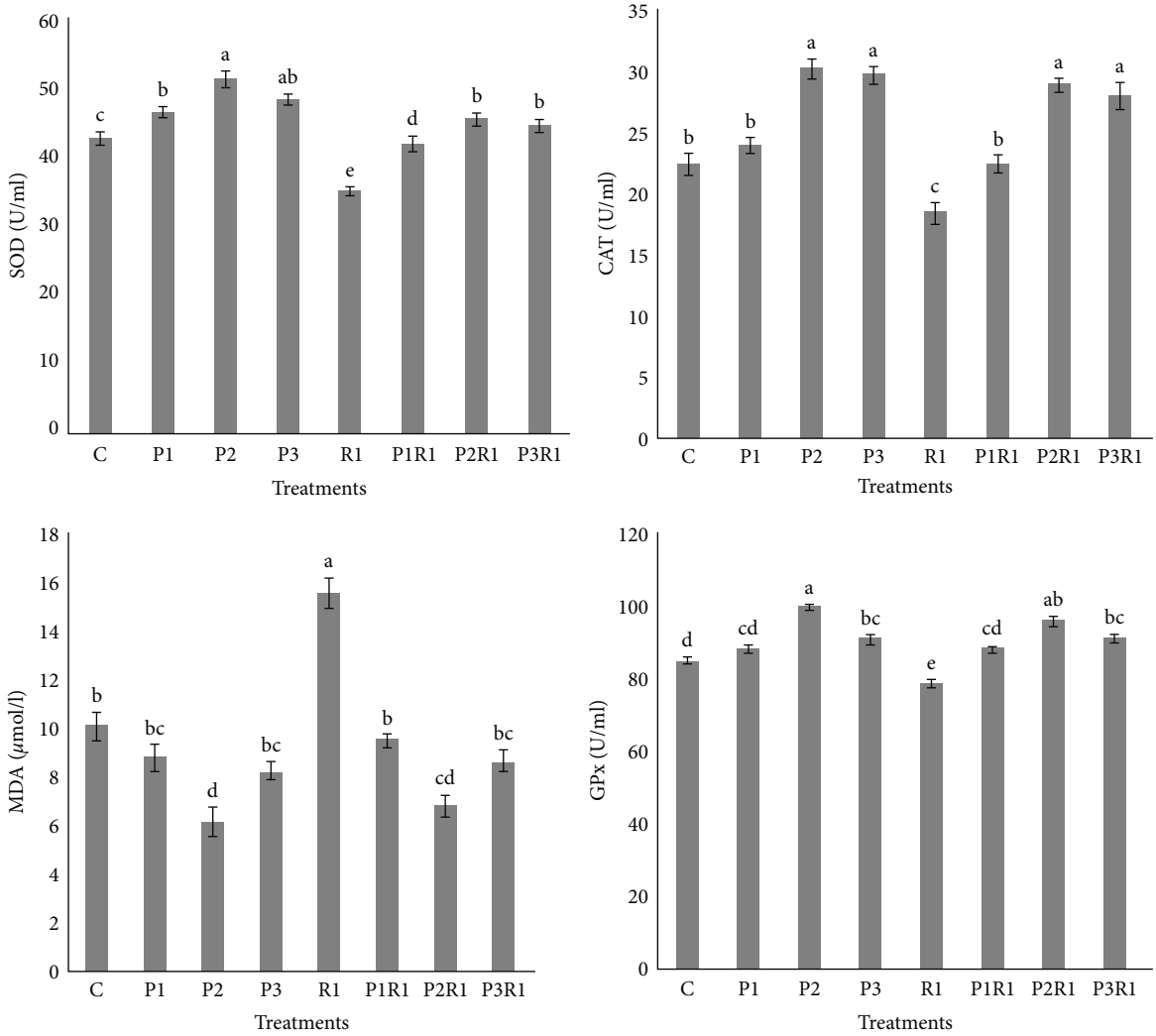
In all assessed antioxidative activity parameters, P-CA and Ronstar separately had a significant ($P<0.05$) effect, but there was a significant interactive effect for MDA and GPx as well.

4. Discussion

In recent years, demands for agricultural food have forced the producers to extremely rely on herbicides in the corpse fields [58]. One of the most widely used herbicides is called Ronstar, with oxadiazon as an active compound that contaminates water resources used for aquaculture. Since the deleterious effects of Ronstar on the environment have been reported in many studies [59–61], here, we used antioxidant activity, immunological, and biochemical assessments to evaluate the possible positive effects of P-CA as a food additive in rainbow trout, one of the most commercially important fish species in aquaculture [62], exposed to Ronstar.

The antioxidative effects of polyphenols, including P-CA, have been studied in numerous researches [63–66]. Our evaluations of antioxidative activity, including DPPH% inhibition percentage, ABTS radical scavenging activity (%), and superoxide anion radical (%) scavenging activity, highly confirmed the antioxidant activity of P-CA used in the present study. The mitigating action of polyphenols (e.g., P-CA) on reactive oxygen species (ROS)-induced toxicity of herbicides may be exerted through neutralizing ROS by donating electrons to them, stimulating endogenous antioxidant enzymes including SOD, CAT, and GPx [67], modulating of multiple signaling pathways involved in ROS production and antioxidant defense [68] and chelating of transition metal ions like iron and copper which are responsible for catalyzing the formation of ROS [69].

The growth performance (FW, WG, and FCR) of the fish increased in P-CA-supplemented treatments. This might be due to the better general biological performance of the fish derived from antimicrobial, antioxidative, and digestive enzyme stimulating effects of P-CA confirmed in our study. Our results were also in consistent with a few previous researches [19, 70]. Oxadiazon has shown negative effects on growth performance in previous studies in common carp and catfish (*Clarias gariepinus*), most likely due to a reduction in fish appetite, dysfunction in metabolism, and energy loss to overcome the herbicide-induced stress [71–73].



	Two-way ANOVA (*P*-value)			
	SOD (U/ml)	CAT (U/ml)	MDA (μmol/l)	GPx (U/ml)
p-CA	0.001	0.001	0.001	0.001
Ronstar^R	0.002	0.002	0.001	0.004
Ronstar^R× p-CA	0.192	0.353	0.000	0.028

Figure 3: Effect of P-CA (p-Coumaric acid) on antioxidant enzymes in rainbow trout exposed to Ronstar. C: negative control, P1: 0.5 g P-CA/kg, P2: 1 g P-CA/kg, P3: 1.5 g P-CA/kg; R1: 12.5% Ronstar toxin, P1R1: 12.5% Ronstar toxin + 0.5 g P-CA/kg, P2R1: 12.5% Ronstar toxin + 1 P-CA/kg, P3R1: 12.5% Ronstar toxin + 1.5 P-CA/kg. Two-way ANOVA of the effect of P-CA (p-Coumaric acid) and Ronstar on antioxidant enzymes in rainbow trout ($P < 0.05$).

In the present study, P-CA successfully retrieved the growth performance in the Ronstar exposed fish. Although P-CA is found to have inhibitory effects on pancreatic lipase [74], there are reports of positive effects of dietary P-CA on lipase and amylase activity [70], which is inconsistent with our findings here. There is not enough evidence about the effects of Ronstar on digestive enzymes, although the activity of lipase, protease, and amylase was enhanced in the intestine, stomach, and other tissues of *Anabas testudineus*, *Heteropneustes fossilis*, and *Oreochromis niloticus* exposed to almix (another organochloride herbicide) [75]. In the current study, Ronstar reduced the activity of these three enzymes, and P-CA could compensate for these Ronstar-derived drops, which might lead to better growth performance.

Biochemical parameters of the fish blood in this study were cortisol, glucose, TP, albumin, and globulin. Although there is not any report on the effects of P-CA in the biochemical parameters of the blood, other polyphenolic compounds have been investigated in a few studies. It was reported the dietary use of commercially available polyphenol (PMIX) had no significant effects on any of the biochemicals (cholesterol, glucose, triglyceride, albumin, and TP)

measured in Asian seabass (*Lates Calcarifer*) [76]. Here, P-CA was effective in TP and glucose levels, while the levels of globulin, albumin, and cortisol were constant. On the other hand, besides glucose and TP, albumin was also affected by Ronstar. The TP and glucose levels were significantly improved by the use of P-CA while the fish was exposed to Ronstar. There are reports on the hepatoprotective effects of P-CA. The activities of ALT, AST, and ALP increased with the administration of Adriamycin in rats showing liver damage, and P-CA treatment reversed the changes [77]. Similar results of hepatoprotective effects of P-CA were observed in acute liver injury caused by CCL^4 in rats [78]. Organochloride herbicides are considered hepatotoxins that are involved in the elevation of liver enzymes [79, 80]. Our findings strongly confirmed the hepatotoxin properties of Ronstar and the hepatoprotective effect of P-CA in rainbow trout.

The humoral immunity of fish is often assessed by measuring the blood immunoglobulin M level [81]. Serum immunoglobulin M can be referred to as an antibody because it is related to the number of molecules produced by B lymphocytes to combat the effects of breakouts [82]. The previous studies on humoral immunity alteration in rats [29] and common carp [70], treated with P-CA, reported an increase in serum total Ig levels, as we observed in the present study. Some studies have reported the negative effects of organochlorine herbicides in different organisms [83–87]. Ronstar effects on immunoglobulins and other humoral immunity parameters were investigated in African catfish (*Clarias gairepinus*); the study reports significant decreases in Ig levels in serum in fish exposed to Ronstar due to impaired protein synthesis, tissue damage, and/or cellular apoptosis [11]. The negative effects of Ronstar on total Ig levels in this study were compensated by P-CA. All other humoral immunity parameters (lysozyme, C3, C4, ACH_{50}, NBT, and MPO) showed almost the same trend of changes as total Ig levels, suggesting the tremendously positive effects of P-CA in immunity enhancement and its potential use in contorting the problems raised by the toxin.

There is not any report on the effects of P-CA on mucus immunity parameters; however, a bunch of studies have addressed the effects of other polyphenols on the innate and mucus immunity in fishes, and all were in consensus that polyphenols were able to induce the innate immunity and there is a positive relationship between the innate and mucus immunity [88–92]. The immunity parameters of the mucus, including lysozyme, ALP, peroxidase, protease, and total Ig, support the increase in mucus immunity and its positive relationship with innate immunity by the use of P-CA, and the defects caused by Ronstar were reduced.

It is shown that feeding common carp fingerlings with rosemary leaf powder significantly raised serum CAT and SOD activity [19]. Additionally, *Carassius auratus*'s gastrointestinal enzymatic antioxidant capacity and CAT, SOD, and GPx were successfully increased by dietary curcumin supplementation [93]. The blood and mRNA levels of antioxidant-related components (CAT, SOD, GR, GPx, and GST) elevated in common carp fed P-CA [19]. The protective effects of P-CA against oxidants and hyperlipidemia are also reported by *in vitro* and *in vivo* researches [94]. There have been many reports that organochlorine herbicide induced oxidative stress in organisms [95–97]. In the present study, the measurements of the antioxidant parameters (SOD, CAT, and GPx) and MDA content suggested that the oxidative stress caused by Ronstar was remediated by the antioxidant activity of P-CA, which was in line with the previous findings.

5. Conclusion

In this study, the total trend of changes in digestive enzymes (lipase, Amylase, protease), growth indices (FW, WG, and FCR), all humoral immunity parameters (total Ig levels, lysozyme, C3, C4, ACH_{50}, NBT, MPO) as well as mucus immunity (lysozyme, ALP, peroxidase, protease, and total Ig), antioxidant parameters (SOD, CAT, GPx, and MDA), liver enzyme (ALT, AST, ALP, and LDH), showed the positive effects of P-CA and negative effects of Ronstar. The only parameters that were not affected by P-CA were globulin, albumin, and cortisol, but Ronstar had its negative effects in these three parameters. The treatments containing both P-CA and Ronstar revealed that the positive effects of dietary P-CA could remediate the negative effects of Ronstar in rainbow trout in a dose-dependent manner; however, further investigations are needed to obtain an optimum level of P-CA. Finally, it is suggested that the present study be strengthened by performing histological studies. As a suggestion, the histopathological effects of the Ronstar on the liver can help to understand the relationship between the physiological changes and tissue alternations.

Data Availability

The data that support the findings of this study are available upon reasonable request to the corresponding authors.

Ethical Approval

All applicable guidelines for the care and use of animals were followed according to the national ethical framework for animal research in Iran. All experiments were performed following the protocol approved by the Committee of Ethics of the Faculty of Sciences, University of Tehran (357; November 8, 2000).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jianbin Ye and Shiyou Yang have done the supervision, conceptualization, resources, funding acquisition, project administration, and writing—review and editing. Somayeh Taheri has done the writing—original draft, formal analysis, and methodology.

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