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Toxicological Effects of Synthesized Pyrazoline Derivative 4-(3-(4-Bromophenyl))-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) Benzenesulfonamide, Compound B8 on Antioxidant Enzymes and Hematological Parameters of Rainbow Trout Alevins (*Oncorhynchus mykiss*)

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The toxicological effects of a synthesized pyrazoline derivative 4-(3-(4-bromophenyl))-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) benzene sulfonamide, compound B8, on rainbow trout (*Oncorhynchus mykiss*) were evaluated by following the activity of the antioxidant enzymes and the hematological parameters. Fish were exposed to variant doses (0.5, 1, and 2.5 mg L^{-1}) of the compound B8 for 48 h. Antioxidant enzymes and activities were measured, and the obtained data were statistically analyzed. Our results showed that concentration at 1 mg L^{-1} significantly increased the malondialdehyde (MDA) levels ($p < 0.05$) in the liver and gills of rainbow trout (*Oncorhynchus mykiss*), while glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were significantly decreased. The increase in MDA levels in the liver and gill could be related to tissue damage induced by oxidative stress after exposure to compound B8. Significant increases in white blood cell count (WBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentrations (MCHC) were obtained ($p < 0.05$). Moreover, significant decreases were observed in erythrocyte numbers, hemoglobin concentrations, hematocrit levels, and platelet numbers ($p < 0.05$). The results showed that exposure to high doses (2.5 mg L^{-1}) of the compound B8 caused oxidative stress in the liver and gills of fish and may lead to acute anemia.

Keywords: antioxidant enzymes, malondialdehyde, pyrazoline, glutathione peroxidase

Introduction

Natural chemical compounds cored with pyrazoline rings have been of high interest to medicinal research in recent decades. Pyrazoline nuclei and their derivatives have a variety of biological activities that are strengthened and extended after modifying the central ring to substitute aromatic or aliphatic groups.[1] A heterocyclic five-membered pyrazoline ring with two adjacent nitrogen atoms could be synthesized to produce pyrazoline derivatives. Research studies[2,3] report pharmaceutical and medicinal applications for natural and synthetic pyrazoline-based compounds. Recently, anti-inflammatory, antioxidant and antitumor activities have been reported for pyrazoline derivatives.[4-6] On the other hand, oxidative stress, antioxidant defense, and antioxidant capacity of biological systems could be enhanced by natural products and synthetic pyrazoline derivatives.[7,8] Evaluation of the antioxidant activities of pyrazoline derivatives and their mechanistic physiology that postpones oxidative stress is highly significant in understanding the chemistry of antioxidant enzymes. Evaluation of the antioxidant enzymes includes the determination of reactive oxygen species (ROS) that

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depend on their activity in the removal of ROS generated after the chemical stress. Studies[9-12] suggest a role for ROS in inducing oxidative damage in different tissues. The biological effects of pyrazolines can be studied by examining their *in vivo* antioxidant enhancements and their capability of inducing oxidative stress within experimental models.[9,10] Cellular oxidative damage is theorized to be involved in the pathogenesis of several diseases and is accompanied by the loss of antioxidant defense capacity.[11] Accordingly, antioxidant enzymes were used as biomarkers for the antioxidant defense response and the occurrence of oxidative damage.[12-14] Oxidative stress parameters and antioxidant enzyme activity measurements after exposure to a therapeutic agent or synthetic compounds are highly significant in the evaluation of drug adverse effects. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) have been used as biomarkers to evaluate the oxidative stress response of natural and synthetic pyrazoline in tissues after exposure to these compounds.[7,15] The aim of this research is to evaluate the biological potential and prospective side effects of compound B8 to support prospective therapeutic and pharmaceutical uses by analyzing antioxidant enzymes and hematological parameters of rainbow trout alevins.

Experimental

Compound synthesis

Pyrazolines were synthesized according to the methods described previously.[16,17] A mixture of a suitable chalcone and *p*-hydrazinobenzenesulfonamide hydrochlorid (Sigma-Aldrich, Schnelldorf, Germany) in 1:1.1 mol ratio in 25 mL ethanol in the presence of glacial acetic acid (0.05 mL) were mixed before refluxing for 19 h to produce compound B8 (Figure 1).

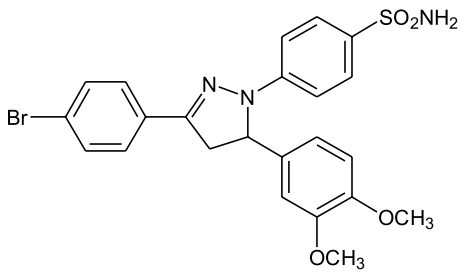

Figure 1. Synthesized pyrazoline derivative 4-(3-(4-bromophenyl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamide (compound B8).

Reactions were monitored by thin layer chromatography (TLC) using chloroform:methanol (4.8:0.2) as a solvent system. The obtained precipitate was filtered and dried before recrystallization in ethanol. The chemical structure of the compound B8 was determined by ^{1}H nuclear magnetic resonance (NMR) (400 MHz) and ^{13}C NMR (100 MHz) spectroscopies using a Varian Mercury Plus spectrometer (Varian Inc., Palo Alto, California, USA). Chemical shifts (δ) are reported in ppm and coupling constants (J) are expressed in hertz (Hz). Mass spectra of the compound B8 were undertaken on an HPLC-TOF Waters Micromass LCT Premier XE (Milford, MA, USA) mass spectrometer using an electrospray ion source (ESI). Melting points were determined using an Electrothermal 9100/IA9100 instrument (Bibby Scientific Limited, Staffordshire, UK) and are uncorrected. The reactions were monitored using silica gel HF254-366 TLC plates (Merck, Darmstadt, Germany).

4-(3-(4-Bromophenyl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamide (B8)

Light green color solid, mp 191-193 °C, yield 38%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 (d, J 8.4 Hz, 2H, Ar-H), 7.62 (d, J 8.8 Hz, 2H, Ar-H), 7.57 (d, J 8.8 Hz, 2H, Ar-H), 7.08 (d, J 8.8 Hz, 2H, Ar-H), 7.02 (s, 2H, -SO_2NH_2), 6.91 (d, J 2.0 Hz, 1H, Ar-H), 6.85 (d, J 8.4 Hz, 1H, Ar-H), 6.66 (dd, J 8.0, 2.0 Hz, 1H, Ar-H), 5.54 (dd, J 12.0, 5.7 Hz, 1H, H-5 (pyrazoline)), 3.90 (dd, J 17.7, 12.0 Hz, 1H, H-4 (pyrazoline), 3.69 (s, 3H, -OCH_3), 3.67 (s, 3H, -OCH_3), 3.17 (dd, J 17.7, 5.7 Hz, 1H, H-4 (pyrazoline); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.8, 149.4, 148.8, 146.6, 134.5, 133.9, 132.4, 131.8, 128.6, 127.8, 123.1, 118.1, 112.9, 112.8, 110.4, 63.2 (C-5 pyrazoline), 56.2 (-OCH_3), 56.1 (-OCH_3), 43.5 (C-4 pyrazoline); HRMS (ESI-MS), calcd. for $C_{23}H_{23}N_3O_4SBr$, $[M + H]^+$: 516.0593; found: 516.0598.

Fish exposure and hematological analysis

Assessment and evaluation of the antioxidant activity and oxidative stress associated with the compound B8 were carried out at the Aquarium Fish Rearing unit of the Aquaculture Faculty at Atatürk University. A total of 120 fish weighing 25 ± 2 g were placed in six glass aquariums with 45 × 50 × 75 cm dimensions. Fishes were fed twice a day with a commercial feed with 45% protein at a rate of 2% body weight. The aquariums were siphoned daily to remove the feed and fecal residues. The experiment water had a pH 7.0, dissolved oxygen 8-9 mg L^{-1}, temperature 11 ± 1.5 °C, and total hardness 220 mg L^{-1}. After 14 days of acclimation, the fish were exposed to different concentrations of compound B8. Experimental doses and exposure times were applied according to Ucar *et al*.[5] The following concentrations of compound B8; 0.00, 0.25, 0.5, 1 and

2.5 mg L^{-1} were prepared and used to expose the fish for 48 h. The first step was to prepare stock solutions by dissolving the compound B8 in dimethyl sulfoxide (DMSO) for the administration of the corresponding dose. The fish were divided into six groups and administered compound B8 at doses of 0.00, 0.25, 0.5, 1, and 2.5 mg L^{-1} (groups 1-5) for 48 h. The control group was exposed to 1 mL DMSO in two replications. The concentrations determined in the stock solutions were added to the aquatic environment and the experiment was carried out under semi-static conditions. Aquariums were monitored, and feces were removed by siphoning. Fish mortalities were recorded daily. The collected fish were placed in clean plastic bags and transferred to the laboratory. Blood samples from all groups were collected after 48 h for hematological analysis. Blood samples of each fish were taken into two different tubes, one containing tripotassium ethylenediaminetetraacetic acid (EDTA) to evaluate the hematological profile, including red blood cell (RBC), white blood cell (WBC), platelets (PLT), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Analysis of RBC, WBC, PLT, Hb, HCT, MCV, MCH, and MCHC were performed with the Beckman Coulter Hematology Analyzer using the Coulter principle of electrical impedance (Beckman Coulter Life Sciences, Indianapolis, USA). Each fish was washed with distilled water, and for each fish sample, weight and length were recorded before the organs were taken and dissected. The liver and gill tissues of the fish were taken and kept at −86 ºC for further analysis.

Oxidative stress enzymatic assays

All chemicals and reagents used in oxidative stress enzymatic assays were of analytical grade. Oxidative stress enzyme and antioxidant enzyme activities, including SOD, CAT, GPx, among liver and gill homogenates, were carried out. The malondialdehyde (MDA) levels and the total protein were determined in the liver and gill homogenates. Tissue homogenates were obtained by taking the whole fish liver and whole gill and homogenizing each organ of each fish in ice-cold phosphate buffer (pH 7.0). The homogenates were centrifuged at 2000 rpm for 10 min at 4 °C. The obtained supernatants were taken for enzymatic assays and another analytical procedure. The protein concentration of homogenates was determined spectrophotometrically according to the procedure described by Bradford.[18] Bovine serum albumin standard was used to determine the protein concentration. MDA is a natural byproduct of cellular lipid damage. The MDA assay was performed by taking 200 µL of the prepared homogenate, 800 µL of phosphate buffer, 25 µL of synthetic antioxidant (BHT) and 500 µL of 30% trichloroacetic acid (TCA) and mixed for a short time before incubated at − 20 °C for 2 h. The mixture was centrifuged at 2000 rpm for 15 min and 1 mL of supernatant was taken and mixed with 75 µL EDTA-Na_2H_2O before adding 250 µL thiobarbituric acid (TBA) and incubate in a water bath for 15 min at 90 °C with a gentle vortex. The pink MDA-TBA colored complex formed by TBA and MDA can be quantified spectrophotometrically at 532 nm in µmol of MDA equivalent. SOD activity was assayed by measuring the color absorbed at 560 nm with nitro-blue tetrazolium (NBT) of superoxide radicals released by xanthine oxidase in the presence of xanthine.[19] The reaction is based on the reduction of the highly water-soluble NBT with superoxide anions (O_2^-) to form water-soluble formazan dye. The competition between SOD and nitro blue tetrazolium (NBT) for (O_2^-) radicals was the basis for the SOD assays. The degree of inhibition of the reaction by SOD is determined calorimetrically. CAT activity was determined according to the method of Aebi.[20] Tissue homogenate supernatant (100 µL) was mixed with an equal volume of absolute alcohol. The mixture was incubated for 30 min in an ice bath for the degradation of inactive CAT and the release of active CAT enzyme. The ice incubated tubes were brought back to room temperature before the addition of 10 µL of Triton X-100. 50 µL of the sample mixture was taken to a cuvette containing 200 µL of phosphate buffer and 250 µL of 0.066 M H_2O_2 as substrate, followed by mixing thoroughly before measuring the decrease in absorbance at 240 nm for 30 s. A molar absorptivity of 43.6 M cm^{-1} was used to calculate CAT activity, one unit of which is equal to the 1 µmol of hydrogen peroxide degraded *per* min *per* mg of protein. GPx was determined by mixing tissue homogenate supernatant (100 µL) with 1 mL reaction mixture containing potassium phosphate buffer, 0.1 M, pH 7.0; 0.2 mM nicotinamide adenine dinucleotide (NADPH); 4 mM glutathione reductase (GSH); 4 mM EDTA; 4 mM sodium azide; and an appropriate amount of enzyme GPx, 0.02 mL. The reaction mixture was incubated at 37 °C for 10 min after the addition of *t*-butyl hydroperoxide to start the reaction. Blank was prepared without adding *t*-butyl hydroperoxide. The rate of reaction was measured at 37 °C by following the decrease in the absorbance at 340 nm for 30 s.

Statistical analysis

Data from biochemical analyses were subjected to analysis of variance (ANOVA) using SPSS 17 (Statistical Package for Social Sciences Program, version 17).[21] Means were compared by using Duncan tests at alpha = 0.05.

Results

The mortality rate was calculated after 24 and 48 h of treating the rainbow trout alevins with different concentrations of compound B8. The mortality rate among rainbow trout alevins after being subjected to different concentrations of compound B8 shows that fish exposed to the lowest concentration (0.25 mg L^{-1}) and highest concentration (2.5 mg L^{-1}) show 50% survivals post 48 h exposure compared to zero deaths in both controls and DMSO treated groups. However, the mortality rate among fish subjected to 0.5 and 1 mg L^{-1} concentrations was 37.5 and 62.5%, respectively. Therefore, 1 mg L^{-1} of compound B8 revealed the highest rate compared with the other three groups. The effect of the compound B8 on the oxidative stress biomarkers and antioxidant enzyme activity obtained in this research is summarized in Figures 2 and 3. Different doses of compound B8 (between 0.25 and 2.5 mg L^{-1}) were used to investigate the effect of pyrazoline derivative on the liver antioxidant enzymes (Figure 2).

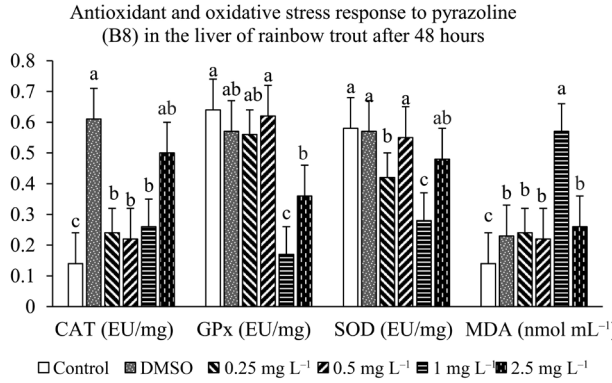

Figure 2. Antioxidant and oxidative stress response to the synthesized pyrazoline derivative (compound B8) in the liver of rainbow trout after 48 h of exposure. Values are represented as mean ± standard deviation (n = 10). Means in the same group with different superscripts (a, b and c) differ significantly at $p < 0.05$.

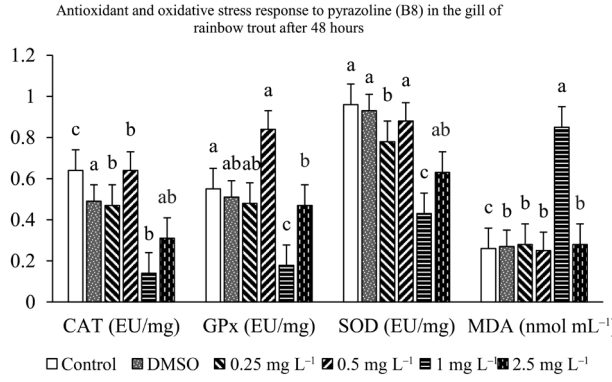

Figure 3. Antioxidant and oxidative stress response to pyrazoline derivative (B8) in the gill of rainbow trout after 48 h exposure. Values are represented as mean ± standard deviation (n = 10). Means in the same group with different superscripts (a, b and c) differ significantly at $p < 0.05$.

The activity of CAT, GPx, and SOD in liver and gill homogenates fluctuated with different concentrations of the compound B8.

Fishes exposed to different concentrations of pyrazoline derivative do not reveal any dose-dependence in the activity of CAT. At lower doses of 0.25 and 0.5 mg L^{-1}, enzyme activity was biased toward the control group's level, whereas, for higher doses, CAT activity was around that for the DMSO control group. On the other hand, the mg L^{-1} dose of the compound B8 had an adverse effect on GPx and SOD activity. A significant decrease in GPx and SOD activities ($p < 0.05$) was obtained for the group treated with 1 mg L^{-1} of compound B8, showing a GPx activity of 0.17 ± 0.10 compared to 0.64 ± 0.07 and 0.57 ± 0.07 in the untreated and DMSO treated control groups, respectively. Similarly, SOD activity of 0.28 ± 0.09 was compared to 0.58 ± 0.06 and 0.57 ± 0.06 for both untreated and DMSO-treated control groups, respectively. However, fish treated with 1 mg L^{-1} of compound B8 showed a significant decrease in CAT (0.26 ± 0.13) compared to the DMSO-treated group (0.6 ± 0.13) ($p < 0.05$) (Figure 2). In the gill tissue, the CAT activity for fish treated with 1 mg L^{-1} of compound B8 decreased significantly to (0.14 ± 0.13) compared to the untreated control (0.64 ± 0.09) and DMSO (0.49 ± 0.09) treated group ($p < 0.05$) (Figure 3). The activity of the other two enzymes of the antioxidant defense system, GPx and SOD, in gill homogenates, decreased significantly compared to the two control groups (Figure 3). GPx activity decreased to 0.178 ± 0.1, whereas the two control groups were 0.55 ± 0.07 and 0.51 ± 0.07 (Figure 3). SOD activity was 0.43 ± 0.14 and the untreated and DMSO control group were 0.96 ± 0.1 and 0.93 ± 0.1, respectively (Figure 3).

Lipid peroxidation assessment was based on the MDA level in the tissue homogenates obtained from fish treated with different concentrations of compound B8. The group treated with 1 mg L^{-1} concentration of compound B8 showed a significant increase ($p < 0.05$) in the level of MDA compared to the untreated control and DMSO control groups as well as the other concentration-treated groups. The MDA level in the homogenates obtained from the gill and liver of fish treated with the concentrations of 0.25, 0.5, and 2.5 mg L^{-1} of compound B8 was nearly similar to the level of MDA found among both untreated controls. However, the MDA level increased significantly in the liver tissue and gill of fish treated with 1 mg L^{-1} compared to the other untreated groups and DMSO control groups (Figure 2).

The evaluation of the impact of compound B8 doses on the hematological parameters showed that WBCs, MCV, and MCH were elevated significantly in the groups

treated with 0.25, 1, and 2.5 mg L^{-1} compared to the two control groups (Table 1) ($p < 0.05$). MCHC level increase was noticed and peak values were reached for 1 mg L^{-1} of compound B8. However, RBCs and Htc were significantly decreased for 0.25, 1, and 2.5 mg L^{-1} compound B8 groups compared to the control groups ($p < 0.05$). Moreover, hemoglobin concentration was significantly decreased for all the doses used except the 0.5 mg L^{-1} dose and platelets count revealed the same pattern obtained for hemoglobin (Table 1).

Discussion

The adverse effect of the synthesized pyrazoline compound 4-(3-(4-bromophenyl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamid (compound B8) on the biological system using rainbow trout alevins as a model was studied. The oxidative stress and antioxidant enzymes could be utilized in order to evaluate the toxic and adverse effects of synthetic chemicals. Apparently, the enzymatic assays for the scavenging enzymes GPx, SOD, and CAT, after exposing the fish to different concentrations of compound B8 failed to enhance oxidative stress in rainbow trout alevins based on the enzymatic activity in the homogenates obtained from the gill and liver tissues. We here expect the poor enhancement of antioxidant enzymes but we cannot deny the overproduction or accumulation of reactive oxygen species that utilize these enzymes in combating ROS and impeding oxidative damage progress.

In this study, we can say that the effect of hormesis was observed in the results obtained independently of the dose of chemical exposure. It is a known condition that exposure to low doses causes hormesis, where it is observed that it compensates for the damage caused by the induction of antioxidant enzymes and helps maintain adaptive processes. Hormesis can be activated due to an imbalance in homeostasis through direct stimulation or through overcompensation.[22,23] The CAT activity determined from DMSO in the research groups was parallel to this situation. Again, the fact that the CAT enzyme was expressed more in the liver tissue may be interpreted as the fact that DMSO induced this expression and could not prevent inhibition in the compound B8 applications and the presence of a possible hormesis effect.

Previous studies[24-26] concluded that the oxidative enzyme GPx activity could be increased following acute exposure to toxicants, with a suggestive adaptive mechanism permitting GSH synthesis, and preventing acute toxic potentials established by oxidative stress. In contrast to the other three concentrations of compound B8 used to expose the rainbow trout, the 1 mg L^{-1} concentration of B8 showed a significant decrease in the activity of the three scavenging enzymes GPx, SOD, and CAT compared with the controls. The results obtained for the MDA for 1 mg L^{-1} concentration of compound B8 increased significantly compared with other concentrations in the study. However, enzymatic activity and MDA levels return to control levels when increasing the concentration of compound B8 to 2.5 mg L^{-1} indicating that a higher concentration of B8 did not affect lipid peroxidation and the antioxidant enzyme activity in the liver and gill of rainbow trout alevins. SOD, which functions as a primary antioxidant enzyme catalyze the conversion of superoxide to H_2O_2 has been analyzed as a marker of physiological effects on cellular macromolecules. It was reported that SOD activities could be increased in response and to protect against an increase in the amount of ROS.[27] We observed no significant difference between SOD activity among treated groups with low and high concentrations compared to the two control groups.

Table 1. Hematological parameters of rainbow trout measured after exposure to compound B8 for 48 h

Hematological parameter	Compound B8				DMSO	Control
	0.25 mg L^{-1}	0.5 mg L^{-1}	1 mg L^{-1}	2. 5mg L^{-1}		
Hb / (g dL^{-1})	9.9 ± 0.78	12.7 ± 0.70	7.63 ± 0.90	8.6 ± 0.78	11.4 ± 0.64	14.4 ± 0.64
RBCs / (10^6 mm^{-3})	1.2 ± 0.2	2.03 ± 0.18	0.87 ± 0.23	1.11 ± 0.20	1.79 ± 0.16	2.50 ± 0.16
WBCs / (10^3 mm^{-3})	188.1 ± 4.5	104. ± 4.0	240.8 ± 5.2	186.1 ± 4.5	103.5 ± 3.6	102.1 ± 3.7
Htc / %	21.63 ± 2.8	32.9 ± 2.6	16.7 ± 3.3	19.8 ± 2.9	28.50 ± 2.3	38.94 ± 2.4
PLT / (10^4 mm^{-3})	8.25 ± 1.1	10.6 ± 1.05	8.00 ± 1.3	9.25 ± 1.2	9.83 ± 0.96	13.00 ± 0.95
MCV / μm^3	178 ± 5.5	163 ± 4.9	193.77 ± 6.4	178 ± 5.4	162 ± 4.4	155 ± 4.5
MCH / pg	82.3 ± 5.3	65.4 ± 4.8	88.2 ± 6.1	86.2 ± 5.3	68 ± 4.3	57.8 ± 4.3
MCHC / (g 100 mL^{-1})	46.1 ± 2.4	39.6 ± 2.2	45.6 ± 2.8	48.2 ± 2.4	41.6 ± 1.99	37.1 ± 2.0

Values are represented as the mean ± standard deviation (n = 10). Means in the same column with different superscripts differ significantly at ($p < 0.05$). DMSO: dimethyl sulfoxide; RBC: red blood cell; WBC: white blood cell; PLT: platelets; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

Elevated ROS agents, including superoxide anion, peroxide radical, hydroxyl radical, and hydrogen peroxide were found to be correlated with reduced antioxidant enzymes level.[26] The reduction in SOD activity after treatment with the 1 mg L^{-1} of compound B8 may refer to the production of oxidants after severe exposure to the chemical stressor. It is well known that the activity of the antioxidant enzyme is interconnected with the reaction product or metabolite and could be influenced directly or indirectly by the activity of another enzyme because of the complexity of the antioxidant pathway. However, there is an assumption indicating that hydrogen peroxide is responsible for low SOD activity compared with the two control groups. An excess of hydrogen peroxide because of over production in the superoxide anion should increase CAT activity, and accumulation in hydrogen peroxide usually increases the activity of CAT and SOD.[15] As illustrated above, the significant decrease in SOD activity in the two tissue homogenates treated with 1 mg L^{-1} of compound B8 could be attributed to the utilization of the over produced ROS in lipid peroxidation. MDA levels were used as a universal biomarker of cellular oxidative stress.[28,29] Moreover, cellular injury induced by ROS leading to elevated MDA levels in both liver and gill homogenates subjected to 1 mg L^{-1} concentration could be explained by either overproduction or accumulation of ROS, given that the MDA level returned to control levels at 2.5 mg L^{-1} concentration of compound B8. A negative correlation has been observed between increasing MDA levels and decreasing SOD activities in previous studies,[15] concluding that the free radical scavenging ability of antioxidant enzymes has decreased with an increase in lipid peroxidation. Oxidative stress is manifested by increased lipid peroxidation and elevated MDA levels. This cannot be entirely attributed to a decrease in the activities of the antioxidant defense system, but various factors may contribute to the development of oxidative stress in a biological system.[30] Non-significant differences in the CAT and GPx activities compared with the control groups were contradicted by the findings of others[7,15] who found that catalase and GPx activities were increased in the homogenates of different tissues treated with synthetic chemical compounds. However, the effects of 2,4-dichlorophenol on the antioxidant system in the liver of freshwater fish were negatively correlated given a decrease in CAT activity compared with the control group.[26]

Hematological profile analysis is an acceptable universal non-specific biological indicator of the physiological statuses in fish because hematological indices in fish can be altered in response to any chemical or physical agents.[31] Some of these alterations in hematological parameters could be a protective mechanism or defense activation towards the physical or chemical stressors. In the present study, the reported decrease in the blood indices (RBC, Htc, Hb, and PLT) after compound B8 treatment indicates fish exposure to chemical stress. This adverse toxic effect led to a significant decrease in the number of erythrocytes, hematocrit, and hemoglobin concentration is consistent with other results reported previously.[31,32]

The decrease in hematological parameters obtained is contradicted by the results reported by Modesto and Martinez,[15] who reported an increase in hematocrit and numbers of erythrocytes after exposing fish to a high concentration of Roundup Transorb® herbicide. The contradicted results refer to the fact that different chemicals have different mechanisms of loading stress on the biological system. Another explanation for the variation in the hematological effects obtained in different studies could be attributed to the type of species used in the study of toxicants.[32] When fish are exposed to toxicants or treated with chemical compounds the total number of white blood cells usually increases.[15,33] Our results showed an absolute increase in the total number of leukocytes in all groups subjected to different concentrations of compound B8. These results obtained for different concentrations could be referred to the activation of the immune defense system in response to the chemical stressors, which in turn may be an adaptive response of the organism to the altered conditions.

Conclusions

Compound B8 was synthesized chemically, and its biological effects were assessed using rainbow trout alevins by following and measuring oxidative stress biomarkers and hematological parameters. The results of the study concluded that oxidative stress-induced cellular damage in the liver and gill tissues correlated with a transient increase in the MDA level, which was attributed to lipid peroxidation. Reduced SOD, CAT, and GPx activity as a compound B8 was shown in this study. The obtained results highlight the need for further studies on the effect of pyrazoline derivatives on antioxidant enzymes and oxidative stress.

Supplementary Information

Supplementary information (Figures S1, S2 and S3) are available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Telat Yanik and Halise Inci Gul was responsible for conceptualization; Dilan Ozmen Ozgun and Halise İnci Gul for data curation and investigation; Arzu Ucar and Gonca Alak for visualization; Muhammed Atamanalp and Esat Mahmut Kocaman for investigation and project administration; Rana Almuhur and Khalid Abu Khadra for validation and visualization; Veysel Pirlak for resources, software, validation; Telat Yanik, Khalid Abu Khadra and Ahmed Maslat for writing original draft, review and editing.

References

1. Ozdemir, A.; Turan-Zitouni, G.; Kaplancikli, Z. A.; *Turk. J. Chem.* **2008**, *32*, 529. [Link] accessed in September 2023
2. Rahman, M. A.; Siddiqui, A. A.; *Int. J. Pharm. Sci. Drug Res*. **2010**, *2*, 165. [Crossref]
3. Ozgun, D. O.; Gul, H. I.; Yamali, C.; Sakagami, H.; Gulcin, I.; Sukuroglu, M.; Supuran, C. T.; *Bioorg. Chem*. **2019**, *84*, 511. [Crossref]
4. Shaharyar, M.; Siddiqui, A. A.; Ali, M. A; Sriram, D.; Yogeeswari, P.; *Bioorg. Med. Chem. Lett*. **2006**, *16*, 3947. [Crossref]
5. Ucar, A.; Ozgun, D. O.; Alak, G.; Gul, H. I.; Kocaman, M.; Yamalı, C.; Parlak, V.; Atamanalp, M.; Maslat, A.; Almuhur, R.; Yanık, T.; *In Vitro Cell. Dev. Biol.: Anim.* **2021**, *57*, 17. [Crossref]
6. Ozgun, D. O.; Alak, G.; Ucar, A.; Bilginer, S.; Gul, H. I.; Kocaman, M.; Yamali, C.; Parlak, V.; Atamanalp, M.; Maslat, A.; Yanik, T.; *Pak. J. Zool*. **2021**, *53*, 2083. [Crossref]
7. Hegde, H.; Ahn, C.; D, S.; Gaonkar, S. L.; Shetty, N. S.; *J. Korean Chem. Soc*. **2017**, *61*, 291. [Crossref]
8. Babu, V. H.; Sridevi, C.; Joseph, A.; Srinivasan, K. K.; *Indian J. Pharm. Sci*. **2007**, *69*, 470. [Crossref]
9. Luo, Y.; Su, Y.; Lin, R. Z.; Shi, H. H.; Wang, X. R.; *Chemosphere* **2006**, *65*, 1064. [Crossref]
10. Afzal, M.; Mansoor, S.; *Asian J. Agric. Sci.* **2012**, *4*, 149. [Link] accessed in September 2023
11. Chen, S.; Fang, A.; Zhong, Y.; Tang, J.; *Arabian J. Chem*. **2022**, *15*, 103561. [Crossref]
12. Abu Khadra, K.; Khalil, A.; Abu Samak, M.; Aljaberi, A.; *Electromagn. Biol. Med.* **2015**, *34*, 74. [Crossref]
13. Arteel, G. E.; Sies, H.; *Environ. Toxicol. Pharmacol.* **2001**, *10*, 153. [Crossref]
14. Halliwell, B.; Chirico, S.; *Am. J. Clin. Nutr*. **1993**, *57*, 715. [Crossref]
15. Modesto, K. A.; Martinez, C. B. R.; *Chemosphere* **2010**, *81*, 781. [Crossref]
16. Bozkurt, E.; Gul, H. I.; Mete, E.; *J. Photochem. Photobiol., A* **2018**, *352*, 35. [Crossref]
17. Yamali, C.; Gul, H. I.; Ece, A.; Taslimi, P.; Gulcin, I.; *Chem. Biol. Drug Des.* **2018**, *91*, 854. [Crossref]
18. Bradford, M. M.; *Anal. Biochem*. **1976**, *72*, 248. [Crossref]
19. Sun, Y.; Oberley, L. W.; Li, Y.; *Clin. Chem*. **1988**, *34*, 497. [Crossref]
20. Aebi, H.; *Methods Enzymol*. **1984**, *105*, 121. [Crossref]
21. *Statistical Package for Social Sciences Program (SPSS*), v.17; SPSS Inc., Chicago, USA, 2008.
22. Calabrese, E. J.; *Pharmacol Res*. **2016,** *110*, 242. [Crossref]
23. Calabrese, E. J.; *Pharmacol Res*. **2016**, *110*, 265. [Crossref]
24. Regoli, F.; Principato, G.; *Aquat. Toxicol*. **1995**, *31*, 143. [Crossref]
25. Otto, D. M.; Moon, T. W.; *Pharmacol. Toxicol*. **1995**, *77*, 281. [Crossref]
26. Zhang, J.; Shen, H.; Wang, X.; Wu, J.; Xue, Y.; *Chemosphere* **2004**, *55*, 167. [Crossref]
27. Otto, D. M.; Moon, T. W.; *Arch. Environ. Contam. Toxicol.* **1996**, *31*, 141. [Crossref]
28. Cerutti, P. A.; *Lancet* **1994**, *344*, 862. [Crossref]
29. Seto, H.; Akiyama, K.; Okuda, T.; Hashimoto, T.; Takesue, T.; Ikemura, T.; *Chem. Lett*. **1981**, *52*, 707. [Crossref]
30. van der Oost, R.; Beyer, J.; Vermeulen, N.; *Environ. Toxicol. Phar.* **2003**, *13*, 57. [Crossref]
31. Shahi, J.; Singh, A.; *Rev. Inst. Med. Trop. São Paulo* **2011**, *53*, 259. [Crossref]
32. Svoboda, M.; Luskova, V.; Drastichova. J.; Ilebek, V.; *Acta Vet. Brno* **2001**, *70*, 457. [Crossref]
33. Elahee, K. B.; Bhagwant, S.; *Ecotoxicol. Environ. Safe.* **2007**, *68*, 361. [Crossref]

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