Influence of Subacute Treatment of Some Plant Growth Regulators on Serum Marker Enzymes and Erythrocyte and Tissue Antioxidant Defense and Lipid Peroxidation in Rats

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ABSTRACT: This study aims to investigate the effects of the plant growth regulators (PGRs) (2,3,5-triiodobenzoic acid (TIBA), Naphthaleneacetic acid (NAA), and 2,4-dichlorofenoxyacetic acid (2,4-D)) on serum marker enzymes (aspartate aminotransferase (AST), alanin aminotransferase (ALT), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH)), antioxidant defense systems (reduced glutathione (GSH), glutathione reductase (GR), superoxide dismutase (SOD), glutathione-S-transferase (GST), and catalase (CAT)), and lipid peroxidation content (malondialdehyde = MDA) in various tissues of rats. 50 and 100 ppm of PGRs as drinking water were administered orally to rats (Sprague–Dawley albino) ad libitum for 25 days continuously. The PGRs treatment caused different effects on the serum marker enzymes, antioxidant defense systems, and the MDA content in experimented rats compared to controls. Results showed that TIBA caused a significant decrease in serum AST activity with both the dosage whereas serum CPK was significantly increased with 100 ppm dosage of TIBA. Meanwhile, serum AST, CPK, and LDH activities were significantly increased with both dosage of NAA and 2,4-D. The lipid peroxidation end-product MDA significantly increased in the all tissues treated with both dosages of PGRs without any change in the brain and erythrocyte of rats treated with both the dosages of 2,4-D. The GSH depletion in the kidney and brain tissues of rats treated with both dosages of PGRs was found to be significant. Furthermore, the GSH depletion in the erythrocyte of rats treated with both dosages of PGRs except 50 ppm dosage of 2,4-D was significant too. Also, the GSH level in the liver was significantly depleted with 50 ppm of 2,4-D and NAA, whereas the GSH depletion in the same tissue did not significantly change with the treatment. The activity of antioxidant enzymes was also seriously affected by PGRs; SOD significantly decreased in the liver, heart, kidney, and brain of rats treated with both dosages of NAA, whereas the SOD activity in the erythrocytes, liver, and heart was either significantly decreased or not changed with two doses of 2,4-D and TIBA. Although the CAT activity significantly increased in the erythrocyte and brain of rats treated with both doses of PGRs, it was not changed in the liver, heart, and kidney. Meanwhile, the ancillary enzyme GR activity significantly increased in the brain, heart, and liver but decreased in the erythrocyte and kidney of rats treated with both doses of PGRs. The drug-metabolizing enzyme GST activity significantly increased in the heart and kidney but decreased in the brain and erythrocytes of rats treated with both dosages of PGRs. As a conclusion, the results indicate that PGRs might affect antioxidant potential enzymes, the activity of hepatic damage enzymes, and lipid peroxidation dose independently. Also, the rats resisted to oxidative stress via antioxidant mechanism but the antioxidant mechanism could not prevent the increases in lipid peroxidation in rat's tissues. These data, along with the determined changes, suggest that PGRs produced substantial systemic organ toxicity in the erythrocyte, liver, brain, heart, and kidney during the period of a 25-day subacute exposure.
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INTRODUCTION

Many chemicals are currently used in agriculture, and plant growth regulators (PGRs) are among those

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widely employed. The amounts of these substances in the environment may soon exceed those of insecticides [1]. Although PGRs are used for pest control and increase in production on a wide variety of crops, little is known about the biochemical or physiological effects in mammalian organisms. There is increased oxidative stress in a polluted area containing high concentrations of polyaromatic hydrocarbons, polychlorinated biphenyls, plant growth regulators (PGRs), and pesticides; however, little is known of how different PGR classes contribute to the oxidative stress induced by these xenobiotics.

Antioxidant defenses, present in all aerobic organisms, include antioxidant enzymes and free-radical scavengers whose function is to remove reactive oxygen species (ROS), thus protecting the functions of organisms from oxidative stress [2]. The sensitivity of cell to oxidants is attenuated by antioxidant defense system such as GSH, GST, CAT, SOD, GPx, GR, and glucose-6-phosphate dehydrogenase (G6PD). The antioxidant defense system maintains a relatively low rate of the reactive and harmful $^{\bullet}OH$. Oxidative stress occurs as a result of the effect of xenobiotics causing disturbances in the antioxidant enzymes system [3]. Among these, GPx, through reduction of both hydrogen peroxide and organic hydroperoxides, provides an efficient protection against oxidative damage and free radicals in the presence of reduced glutathione (GSH). Previously, oxidized GSH is regenerated by GR. SOD catalyzes dismutation of superoxide anion radicals, whereas CAT eliminates hydrogen peroxide. Antioxidant enzymes play a crucial role in maintaining cell homeostasis. Another group of enzymes, GST, acts as a catalyst on a wide variety of conjugation reactions of glutathione with xenobiotic compounds containing electrophilic center. Additionally, there are glutathione-independent enzymes that act as part of the cellular defense system against toxicity originated by active oxygen forms [4]. Oxidative stress may produce DNA damage, enzymatic inactivation, and peroxidation of cell constituents, especially lipid peroxidation when antioxidant defenses are impaired or overcome [5]. Oxidative stress is defined as a loss of the normal balance between reactive oxygen species (ROS) production and the antioxidant system [3]. Indeed, in addition to an excess generation of ROS, xenobiotics have a decreased or increased antioxidant capacity, which causes oxidative damage to cells. Superoxide anion (O_2^-) is a major ROS and can be converted to hydrogen peroxide, which can be used by activated myeloperoxidase (MPO) to produce the strong oxidant hypochlorite [6].

In the literature, it is reported that fecundity, longevity, and egg viability have been changed in different insects by PGRs treatment [7–11]. The findings of Hsiao et al. [12] show that kinetin has effective free-radical scavenging activity in vitro and antithrombotic activity in vivo. Frukawa et al.'s [13] findings indicate that IAA might induce the neuronal apoptosis in the S phase and lead to microencephaly. Also, de Melo et al. [14] determined that incubation for 24 h in the presence of IAA (1 mM) showed increase in the activities of SOD, CAT, and GPx in rat neutrophils and lymphocytes. The addition of exogenous antioxidant enzymes (SOD and CAT) prevented the loss of cell membrane integrity induced by IAA. John et al. [15] observed that IAA possesses teratogenic effects in mice and rats. El-Mofty and Sakr [16] found that gibberellic acid (GA_3) induced liver neoplasm in Egyptian toads, and they suggested that the tumors could be diagnosed as hepatocellular carcinomas. IAA and Kn also affect the hematological and biochemical parameters of rats [17]. Ozmen et al. [18] observed that abscisic acid and gibberellic acid affect sexual differentiation and some physiological parameters of laboratory mice. Also, it is reported that PGRs cause increase in the number of splenic plaque-forming cells and circulating white blood cells, hematocrit values, and thymus weight in young deer mice [19]. On the other hand, some PGRs have been shown to affect the antioxidant defense systems and MDA content [20,21]. The effects of IAA and Kn were also investigated on human serum enzymes in vitro. IAA was found to inhibit aspartate aminotransferase (AST) and activate amylase, creatine phosphokinase (CPK), and lactate dehydrogenase (LDH). Kn inhibited muscle creatine kinase (CK-MB), while it activated AST and alanin aminotransferase (ALT) [22]. On the other hand, we have found that the level of IAA AST, LDH, and CPK was increased significantly by IAA, and the level of AST, LDH, and CPK was increased significantly by Kn in another study [23]. In addition, PGRs may induce oxidative stress, leading to generation of free radicals and cause lipid peroxidation as one of the molecular mechanisms involved in PGRs-induced toxicity [24,25]. According to the US Environmental Protection Agency (EPA), toxic xenobiotic chemicals are irritating to the eyes, skin, and mucous membrane and since it is easily absorbed dermally, orally, or by inhalation, can injure liver, kidney, muscle, and brain tissues.

In spite of reasons stated above, there is still great contradiction between the results. Therefore, in order to achieve a more rational design of PGRs, it is necessary to clarify the mechanism of toxicity for PGRs and develop an understanding of structure–toxicity relationships. With this aim, the treatment of PGRs was done orally because the effect of chemicals represents a well-characterized in vivo toxicity model system. The tissues were chosen because of its important role

during detoxification in degradation and bioactivation of PGRs.

MATERIALS AND METHODS

Materials

PGRs, thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), ethlenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), metaphosphoric acid, 5,5′dithiobis-(2-nitrobenzoic acid) (DTNB), trihydroxymethyl aminomethane (Tris), 1-chloro-2,4-dinitrobenzene (CDNB), oxidized glutathione (GSSG), β-nicotinamide adenine dinucleotide phosphate (reduced) (NADPH), potassium chloride (KCl), hydrogen peroxide (H_2O_2), and sodium chloride (NaCl) of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Kits for antioxidant enzymes analysis were supplied by Randox Laboratories Ltd.

Animals

Rats (Sprague–Dawley albino) 4 months of age with an average weight of 150–200 g were provided by the animal house of the Medical School of Yüzüncü Yil University, and were housed in seven groups, each group containing six rats. The animals were housed at $20 \pm 2^{\circ}$C an in daily light/dark cycle. All animals were fed a group wheat–soybean–meal based diet and water ad libitum in stainless cages, and received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Science and published by the National Institutes of Health [26]. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments.

Treatment of Rats

This investigation was performed on male rats. The rats were exposed to 50 and 100 ppm TIBA, NAA, and 2,4-D ad libitum for 25 days as drinking water. Fifty and hundred milligrams of PGRs were dissolved in 1 mL of 1 N NaOH, and then were diluted with tap water until 1000 mL to obtain a 50 and 100 ppm dose. For the control rats, only 1 mL of 1 mL of 1 N NaOH was added to 1000 mL of tap water. Since all rats have the same physiologic characters, daily water consumption of all groups rat was approximately 28 ± 3 mL during the tests. Consequently, the PGRs intake amount of each rat was about 1.5 ± 0.3 and 3 ± 0.5 mg per day.

At the end of the treatments, the rats were anesthetized by inhalation of diethyl ether, and after blood and tissues samples were obtained, they were sacrificed. The blood samples were obtained from a cardiac puncture using syringe for the determination of serum enzyme levels and biochemical analysis. For serum enzyme levels, blood samples were put immediately into ice-chilled silicon disposable glass tubes. The serum samples were obtained by centrifuging blood samples at $4000 \times g$ for 15 min at 4°C, and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were put immediately into silicon disposable glass tubes with EDTA as an anticoagulant. Blood samples were centrifuged at $4000 \times g$ for 15 min at 4°C, and erythrocyte pellets were obtained. Then the pellets were washed three times with physiological saline (0.9% NaCl). The GSH concentration in erythrocytes and tissues was measured just after the animals were sacrificed because of tremendous loss of GSH. The concentration of GSH and MDA and the activity of SOD, GR, CAT, and GST in erythrocytes were measured in the pellets.

The tissues were dissected and put in Petri dishes. After washing the tissues with physiological saline (0.9% NaCl), samples were taken and kept at −78°C until analysis. The tissues were homogenized for 5 min in 0.115 M ice-cold KCl solution (1:5 w/v) using a glass-porcelain homogenizer (20 kHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at $7000 \times g$ for 15 min. All processes were carried out at 4°C. Supernatants and homogenates were used to determine antioxidant defense systems and MDA.

Biochemical Analysis

The erythrocyte and tissues MDA concentration was determined using the method described by Jain et al. [27], based on the TBA reactivity. Briefly, 0.2 mL erythrocyte pellets or supernatant obtained from tissues, 0.8 mL phosphate buffer (pH 7.4), 0.025 mL BHT, and 0.5 mL 30% TCA were added to the tubes and mixed. After 2 h incubation at −20°C, the mixture was centrifuged ($400 \times g$) for 15 min. After this, 1 mL supernatant was taken and added to each tube, and then 0.075 mL of 0.1 M EDTA and 0.25 mL of 1% TBA were added. These tubes with Teflon-lined screw caps were incubated at 90°C in a water bath for 15 min and cooled to room temperature. The optical density was measured at 532 and 600 nm in a spectrophotometer for erythrocyte MDA, and the optical density was measured at 532 for tissues MDA concentration (Novaspec II Pharmacia-Biotech, Biochrom Ltd., UK).

The erythrocyte and tissues GSH concentration was measured using the method described by Beutler et al. [28]. Briefly, 0.2 mL fresh erythrocyte pellet or supernatant was added to 1.8 mL distilled water. Three milliliter of the precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA, and 30 g NaCl in 100 mL distilled water) was mixed with haemolysate. The mixture was allowed to stand for approximately 5 min and then filtered (Whatman N 42). Two milliliter of filtrate was taken and added into another tube, and then 8 mL of the phosphate solution (0.3 M disodium phosphate) and 1 mL DTNB were added. A blank was prepared with 8 mL of phosphate solution, 2 mL diluted precipitating solution (three parts to two parts distilled water), and 1 mL DTNB reagent. A standard solution of the glutathione was prepared (40 mg/100 mL). The optical density was measured at 412 nm in the spectrophotometer.

CAT (EC 1.11.1.6) activity was determined using the method described by Beutler [29], Briefly, 1 M Tris–HCl, 5 mM EDTA (pH 8), 10 mM H_2O_2, and H_2O were mixed and the rate of H_2O_2 consumption at 240 nm and 37°C was used for quantitative determination of CAT activity (20 μL of 95% ethanol was added in 1 mL of hemolysate to break down complex of catalase and H_2O_2). One EU was defined as the enzyme breaking 1 μmol H_2O_2 per min at 25°C and optimal pH (pH 8.0). GST (EC 2.5.1.18) was assayed at 25°C spectrophotometrically by following the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm as described by Mannervik and Guthenberg [30]. One EU was defined as the enzyme conjugation of 1 μmol of glutathione with CDNB per min at 25°C and optimal pH (pH 7.8). GR (EC 1.6.4.2) activity was assayed at 37°C and 340 nm by following the oxidation of NADPH by GSSG [29]. One EU was defined as the enzyme oxidation 1 μmol of NADPH per min at 25°C and optimal pH (pH 8.0). SOD (EC 1.15.1.1) activity was measured at 505 nm and 37°C and calculated using inhibition percentage of formazon formation [31]. One EU was defined as the enzyme inhibition percentage of formazon formation 1 μmol per min at 25°C and optimal pH (pH 7.8). The EU in supernatant converting to enzymes units/gram tissues was calculated as considered in the dilution factors.

Measurement of Enzyme Levels

Serum enzyme activities (AST (EC 2.6.1.1), ALT (E.C 2.6.1.2), CPK (EC 2.7.3.2), and LDH (EC 1.1.1.27)) were measured by an auto analyzer (BM/HITACHI-911), using the kits.

Analysis of Data

The data were expressed as mean ± standard deviation (SD). For statistical analysis the SPSS/PC+ package (SPSS/PC+, Chicago, IL, USA) was used. For all parameters, means and SD were calculated according to the standard methods. The Mann–Whitney U-test was employed to examine differences between means of the treatments and the control rats. The significance level was accepted at $p \leq 0.05$ for all tests.

RESULTS AND DISCUSSION

In recent years, a significant increase in the use of PGRs against harmful agricultural pests, giving rise to loss in production have been observed in Turkey as well as in the rest of the world. One of the major reasons for the increase is the ease of using PGRs and ensuring an absolute result. In the present study, 2,4-D, TIBA, and NAA were preferred because information on their negative effects on higher animals is very limited for in vivo, oral exposures. Also, NAA, 2,4-D, and TIBA are found in a wide variety of biologically active compounds. The data collected in this study were all from one time point of the experiment. We found that the treatment of NAA, 2,4-D, and TIBA increased some of the activities in serum marker enzymes, the production of lipid peroxides, and affected antioxidant defense of various rat tissues. To find out the significance of biochemical changes in different tissues exposed to 2,4-D, TIBA, and NAA for 25 days, the data have been subjected to Man–Whitney U test.

The results of experiment showed that the treatment of rats with 2,4-D, TIBA, and NAA changed activities of serum enzymes (Table 1), MDA contents, GSH levels, and antioxidant enzymes such as CAT, GST, GR, and SOD in the erythrocyte, liver, kidney and heart in comparison to control rats (Tables 2–6).

So far, no study examining the effect of 2,4-D, TIBA, and NAA in vivo has been made on rat erythrocyte and that of tissues MDA content and antioxidant enzyme activities. Therefore, we could not have a chance to compare our results with the previous results. However, the data obtained for control animals are in accordance with previous studies [20,21,23]. In addition, because of high variability in analyzing immune potential and antioxidant enzymes–chemicals interaction in vivo and in vitro, and inconsistent factors like treatment time and manner, the setting of studies, purity of chemicals, species tissue differences, etc., it is difficult to compare the present data to previous studies regarding the toxicological effect. For the extent of chemical effect too, little is known about the biochemical or physiological effects in the vertebrates.

TABLE 1. Effects of 50 and 100 ppm 2,4-D, TIBA, and NAA on Serum Enzyme Levels of Rats

Dose	*Parameters*	*Control mean ± SD*	*2.4-D mean ± SD*	*TIBA mean ± SD*	*NAA mean ± SD*
50 ppm	AST(U/L)	103.8 ± 28.3	113.8 ± 44.9*	49 ± 18.9*	159.8 ± 86.3*
	ALT (U/L)	41.3 ± 16.2	38.2 ± 5.40	24.1 ± 8.60	48.2 ± 180
	LDH (U/L)	244 ± 111.5	893.5 ± 162.9*	344.2 ± 126.5	1636.5 ± 599.4*
	CPK (U/L)	161.5 ± 73.8	667.7 ± 224.3*	234.2 ± 92.5	1857 ± 473.4*
100 ppm	AST(U/L)	103.8 ± 28.3	191.5 ± 49.80*	52.16 ± 18.21*	196.2 ± 23.6*
	ALT (U/L)	41.3 ± 16.2	36.83 ± 15.09	26.83 ± 5.84	34.16 ± 10.12
	LDH (U/L)	244 ± 111.5	716.83 ± 160.6*	139.33 ± 71.83	1426.2 ± 304.3*
	CPK (U/L)	161.5 ± 73.8	554.5 ± 181.53*	568 ± 80.68*	1144.0 ± 321.4*

Each value represents the mean ± SD.
* $p < 0.05$.

TABLE 2. Effects of 50 and 100 ppm 2,4-D, TIBA, and NAA on Antioxidant Defense and MDA in Erythrocyte of Rats

Dose	*Parameters*	*Control mean ± SD*	*2.4-D mean ± SD*	*TIBA mean ± SD*	*NAA mean ± SD*
50 ppm	GSH mg/dL	39.6 ± 1.3	31.4 ± 3.0	31.7 ± 3.7*	30.25 ± 4.8*
	MDA nmol/mL	1.3 ± 0.3	2.87 ± 1.3	4.0 ± 1.3*	5.04 ± 1.7*
	CAT U/mL	78.1 ± 17.5	193.6 ± 20.9*	154.4 ± 18.6*	203.8 ± 17.6*
	GST U/mL	3.75 ± 0.6	2.6 ± 0.3*	2.2 ± 0.05*	2.1 ± 0.2*
	GR U/mL	2.54 ± 0.33	0.52 ± 0.11*	0.60 ± 0.13*	0.43 ± 0.05*
	SOD U/mL	485.5 ± 167.2	452.5 ± 63.9	496.9 ± 75.5	439.7 ± 50.2
100 ppm	GSH mg/dL	39.6 ± 1.3	21.8 ± 7.4*	23.4 ± 8.6*	27.6 ± 3.5*
	MDA nmol/mL	1.3 ± 0.3	2.94 ± 1.2	4.3 ± 1.3*	3.2 ± 1.1*
	CAT U/mL	78.1 ± 17.5	177.7 ± 44.3*	355.5 ± 76.2*	255.7 ± 44.3*
	GST U/mL	3.75 ± 0.6	2.45 ± 1.03*	2.47 ± 1.08*	2.3 ± 0.9*
	GR U/mL	2.54 ± 0.33	0.3 ± 0.02*	0.39 ± 0.05*	0.59 ± 0.2*
	SOD U/mL	485.5 ± 167.2	443.6 ± 123.4	435.6 ± 132.6	434.5 ± 75.6

Each value represents the mean ± SD.
* $p < 0.05$.

TABLE 3. Effects of 50 and 100 ppm 2,4-D, TIBA, and NAA on Antioxidant Defense and MDA in Liver Tissues of Rats

Dose	*Parameters*	*Control mean ± SD*	*2.4-D mean ± SD*	*TIBA mean ± SD*	*NAA mean ± SD*
50 ppm	GSH mg/g	31.5 ± 3.3	25.6 ± 1.1*	28.3 ± 3.4	24.1 ± 1.5*
	MDA nmol/g	62.8 ± 2.05	89 ± 4.6*	79 ± 9.1*	101 ± 7.9*
	CAT U/g	17.1 ± 2.6	16.0 ± 2.3	13.7 ± 1.1	14.8 ± 3.2
	GST U/g	28.5 ± 1.2	37.8 ± 1.5	33.1 ± 2.3	33.8 ± 1.8
	GR U/g	0.23 ± 0.06	0.23 ± 0.09	0.48 ± 0.15*	0.54 ± 0.18*
	SOD U/g	1986.4 ± 191.9	835.5 ± 420.8*	858.4 ± 479.4*	880.4 ± 86.1*
100 ppm	GSH mg/g	31.5 ± 3.3	28.2 ± 4.1	32.9 ± 7.6	29.5 ± 5.3
	MDA nmol/g	62.8 ± 2.05	86.6 ± 1.5*	102.5 ± 3.5*	129 ± 1.9*
	CAT U/g	17.1 ± 2.6	15.9 ± 2.7	13.9 ± 1.7	14.4 ± 2.3
	GST U/g	28.5 ± 1.2	32.3 ± 1.02	29.6 ± 3.3	32.5 ± 1.2
	GR U/g	0.23 ± 0.06	0.46 ± 0.09*	0.59 ± 0.17*	0.53 ± 0.14*
	SOD U/g	1986.4 ± 191.9	817.6 ± 70.3*	880.4 ± 86.1*	884.8 ± 179.2*

Each value represents the mean ± SD.
* $p < 0.05$.

In the present study, PGRs caused a significant alteration in the serum enzyme activities in rats treated with two different doses of PGRs in comparison to those of controls (Table 1). Namely, TIBA caused a significant decrease in serum AST activity with both of the dosage, whereas serum CPK significantly increased with 100 ppm dosage of TIBA. Meanwhile, serum AST, CPK, and LDH activities significantly increased with both dosage of NAA and 2,4-D. The reasons for such effect of PGRs are not understood at present. However, it is conceivable that PGRs, as a toxicological agent like other pesticides, might be interacting primarily with

TABLE 4. Effects of 50 and 100 ppm 2,4-D, TIBA, and NAA on Antioxidant Defense and MDA in Kidney Tissues of Rats

Dose	*Parameters*	*Control mean ± SD*	*2.4-D mean ± SD*	*TIBA mean ± SD*	*NAA mean ± SD*
50 ppm	GSH mg/g	43.4 ± 3.2	26.9 ± 7.2*	26.8 ± 4.7*	30.3 ± 8.3*
	MDA nmol/g	37.8 ± 6.7	110.9 ± 17.9*	107.3 ± 16.01*	100.6 ± 26.3*
	CAT U/g	28.1 ± 4.1	17.5 ± 3.5	16.3 ± 3.8	16.5 ± 2.6
	GST U/g	6.2 ± 0.9	13.6 ± 2.4*	12.5 ± 0.8*	14.1 ± 2.4*
	GR U/g	1.3 ± 0.1	0.42 ± 0.1*	0.39 ± 0.1	0.42 ± 0.1*
	SOD U/g	323.1 ± 70.6	224.5 ± 53.5	215.7 ± 140.8	249.9 ± 106.7
100 ppm	GSH mg/g	43.4 ± 3.2	19.3 ± 6.3*	25.5 ± 3.5*	22.5 ± 7.1*
	MDA nmol/g	37.8 ± 6.7	102.2 ± 8.6*	91.8 ± 23.8*	82.4 ± 11.7*
	CAT U/g	28.1 ± 4.1	16.3 ± 2.5	18.4 ± 4.7	20.2 ± 4.1
	GST U/g	6.2 ± 0.9	12.8 ± 0.7*	11.4 ± 1.7*	12.6 ± 1.1*
	GR U/g	1.3 ± 0.1	0.26 ± 0.1*	0.34 ± 0.1*	0.21 ± 0.1*
	SOD U/g	323.1 ± 70.6	238.1 ± 192.3	409.0 ± 119.5	408.4 ± 96.6

Each value represents the mean ± SD.
* $p < 0.05$.

TABLE 5. Effects of 50 and 100 ppm 2,4-D, TIBA, and NAA on Antioxidant Defense and MDA in Brain Tissue of Rats

Dose	*Parameters*	*Control mean ± SD*	*2.4-D mean ± SD*	*TIBA mean ± SD*	*NAA mean ± SD*
50 ppm	GSH mg/g	44.9 ± 4.6	12.9 ± 2.2*	17.2 ± 4.4*	12.8 ± 2.5*
	MDA nmol/g	56.1 ± 13.8	38.5 ± 5.2	36.1 ± 4.9	36.1 ± 4.8
	CAT U/g	29.9 ± 3.9	21.5 ± 6.4	11.5 ± 2.7*	12.2 ± 3.5*
	GST U/g	17.5 ± 0.2	11.4 ± 0.7*	11.6 ± 0.9*	11.6 ± 1.4*
	GR U/g	0.20 ± 0.02	0.91 ± 0.14*	0.70 ± 0.1*	0.69 ± 0.1*
	SOD U/g	790.2 ± 152.1	659.4 ± 31.7	521.4 ± 83.1*	529.9 ± 101.4*
100 ppm	GSH mg/g	44.9 ± 4.6	12.7 ± 1.9*	12.9 ± 4.1*	11.8 ± 1.2*
	MDA nmol/g	56.1 ± 13.8	41.7 ± 10.5	38.3 ± 8.4	35.7 ± 6.1
	CAT U/g	29.9 ± 3.9	21.5 ± 4.8*	13.7 ± 1.8*	15.9 ± 5.4*
	GST U/g	17.5 ± 0.2	12.9 ± 0.6*	12.2 ± 0.9*	12.5 ± 0.8*
	GR U/g	0.20 ± 0.02	0.60 ± 0.02*	0.54 ± 0.1*	0.54 ± 0.01*
	SOD U/g	790.2 ± 152.1*	163.4 ± 56.9*	263.8 ± 68.8*	236.6 ± 59.6*

Each value represents the mean ± SD.
* $p < 0.05$.

TABLE 6. Effects of 50 and 100 ppm 2,4-D, TIBA, and NAA on Antioxidant Defense and MDA in Heart Tissue of Rats

Dose	*Parameters*	*Control mean ± SD*	*2.4-D mean ± SD*	*TIBA mean ± SD*	*NAA mean ± SD*
50 ppm	GSH mg/g	65.0 ± 3.9	41.5 ± 11.9	41.2 ± 6.2	31.2 ± 4.4*
	MDA nmol/g	31.9 ± 6.9	58 ± 0.8*	172 ± 5.6*	104 ± 3.6*
	CAT U/g	19.7 ± 3.7	19.2. ± 5.2	20.5 ± 7.5	17.1 ± 2.8
	GST U/g	1.9 ± 0.2	8.1 ± 1.4*	7.8 ± 0.8*	8.6 ± 0.9*
	GR U/g	0.04 ± 0.02	0.32 ± 0.06*	0.38 ± 0.1*	0.35 ± 0.1*
	SOD U/g	984.4 ± 219.4	447.8 ± 109.7*	749.1 ± 69.4	739.3 ± 142.2
100 ppm	GSH mg/g	65.0 ± 3.9	37.8 ± 10.4*	39.9 ± 2.6*	35.2 ± 5.7*
	MDA nmol/g	31.9 ± 6.9	131 ± 6.9*	118 ± 2.9*	47 ± 2.1*
	CAT U/g	19.7 ± 3.7	24.7 ± 6.3	21.5 ± 8.3	23.8 ± 3.4
	GST U/g	1.9 ± 0.2	8.9 ± 0.3*	8.4 ± 1.1*	8.5 ± 0.7*
	GR U/g	0.04 ± 0.02	0.40 ± 0.04*	0.39 ± 0.1*	0.33 ± 0.04*
	SOD U/g	984.4 ± 219.4	640.6 ± 88.6*	762.1 ± 80.5	717.7 ± 91.7*

Each value represents the mean ± SD.
* $p < 0.05$.

the liver and muscle tissue cells' membrane, resulting in structural damage and changes in enzyme leakage and in metabolism of the constituents. PGRs may pressurize this enzyme into plasma as a result of enzyme leakage breakdown. It is known that these enzymes are mainly localized in the cytoplasm. Any damage in hepatic cells may result in alteration in the serum levels. Aminotransferases, because they are very active in the liver and their activity can be detected in very small amounts, are most useful in the monitoring of people exposed to such industrial chemicals. Therefore, damage to liver cell may result in leakage of the

enzymes into the plasma due to a large concentration gradient [32]. Also, it is known that AST, localized both in the cytoplasm and mitochondrion, leakages more into the bloodstream than do ALT when serious cellular damage occurred [33]. Consequently, this study may suggest that any damage in hepatic cells may result in alteration in the serum AST level too. Similarly, another researcher determined that AST and ALT activities in the serum of *Channa striatus* have decreased following exposure to xenobiotics [34]. Oruc and Uner [35] also found an increase in the serum LDH activity in *Cyprinus carpio* following exposure to 2,4-Diamin. On the other hand, Celik et al. [22,23] determined effects of different PGRs on serum marker enzyme fluctuations as in vivo and in vitro studies. Although the treatment, materials of studies, and the setting of studies are different, this result is partly in accordance with our result.

In addition to the fluctuated level of serum marker enzymes, the results of the present study have also demonstrated that the rats treated with both doses of PGRs could have affected the antioxidant defense systems in the vertebrates. This is evidenced from our observation that, upon PGRs treatment in vivo, the concentration of MDA and the antioxidant defense markers in erythrocytes, liver, kidney, and heart differs from that of control rats. The present study showed that the lipid peroxidation end-product MDA significantly increased in almost all tissues treated with both dosages of PGRs without any change in the brain and erythrocyte of rats treated with both the dosages of 2,4-D. The increased MDA content may result from an increase in hydroxyl radicals (•OH). However, it is conceivable that 2,4-D, TIBA, and NAA might be interacting primarily with the erythrocytes, liver, heart, and kidney tissues, resulting in lipid peroxidation processes by the way of increase in superoxide radicals as a result of stressed condition in the rats, leading to an increase in lipid peroxidation. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids, and increased MDA content is an important indicator of lipid peroxidation [36]. Also, it is known that •OH can initiate lipid peroxidation in tissues [37]. Candeias et al. [24] investigated the peroxidation of liposomes by a haem peroxidase and hydrogen in the presence of IAA and derivatives. They found that these compounds can accelerate lipid peroxidation up to 65 times, and this is attributed to the formation of peroxyl radicals that may react with the lipids, possibly by hydrogen abstraction. Also, we determined that while Kn did not affect the levels of MDA erythrocyte, muscle, heart, kidney, and liver tissues, IAA caused a significant increase in the MDA concentration in kidney and liver [20]. Consequently, these studies agree with our previous results although the treatment of time and kind of chemicals are different.

The present study also showed that the GSH depletion in the kidney and brain tissues of rats treated with both dosages of PGRs was significant. Furthermore, the GSH depletion in the erythrocyte of rats treated with both dosages of PGRs except 50 ppm dosage of 2,4-D was significant too. Also, the GSH level in the liver was significantly depleted with 50 ppm dosage of 2,4-D and NAA whereas the GSH depletion in the same tissue did not significantly change with the treatment. Reduced GSH and its metabolizing enzymes provide the major defense against ROS-induced cellular damage [38]. Its depletion might enhance the risk of the oxidative stress [2]. This study showed that GSH is consumed in order to serve the antioxidative protective function. Thus, a considerable decline in GSH content in the tissues under the present experimental model may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from PGRs. The activity of antioxidant enzymes was also seriously affected by PGRs; SOD significantly decreased in the liver, heart, kidney, and brain of rats treated with both doses of NAA whereas SOD activity in the erythrocytes, liver, and heart were either significantly decreased or not changed with two doses of 2,4-D and TIBA. Although CAT activity significantly increased in the erythrocyte and brain of rats treated with both doses of PGRs, it was not changed in the liver, heart, and kidney. Meanwhile, the ancillary enzyme GR activity significantly increased in the brain, heart, and liver but decreased in the erythrocyte and kidney of rats treated with both doses of PGRs. The drug-metabolizing enzyme GST activity significantly increased in the heart and kidney but decreased in the brain and erythrocytes of rats treated with both doses of PGRs. Although the reasons for such effect of PGRs are not understood at present, the increased ROS may result an increase in antioxidant enzymes. It is conceivable that PGRs might be interacting primarily with the tissues, resulting in fluctuated enzyme activities by the way of increased superoxide radicals as a result of stress condition in the rats. However, it is not a general rule that increases in pollutant concentrations induce antioxidant activity. Doyotte et al. [39] pointed out that a decreased enzyme activity's response may accompany a first exposure to pollutants, which can be followed by an induction of antioxidant systems. Thus, the existence of an inducible antioxidant system may reflect an adaptation of organisms. Another possibility is that PGRs may lead to the inhibition or stimulation of the enzymes synthesis in tissues as a result of mRNA breakdown or cellular transcription mechanisms. In contrast, Dimitrova et al. [40] suggested that the superoxide

radicals by themselves or after their transformation to H_2O_2 cause an oxidation of the cysteine in the enzyme and decrease in the SOD activity. The decreased SOD activity may reflect a cellular oxidative stress due to PGRs exposure. According to Halliwell and Gutteridge [37], SOD is considered to be one of the most active enzymes; its activity is sufficient for dismutation of superoxide anions produced during oxidative stress in cells. The lack of differences in SOD activity between the group of frogs treated with pesticides for 12 days and the control group may attest to development of the adaptive mechanism expressed by the SOD activity. The elevated activity of CAT is due to the adaptive response to the generated free radicals. The enzymatic antioxidants such as SOD, GR, GST, and CAT have been shown to be sensitive indicators of increased oxidative stress in *Mugil* sp. obtained from a polluted area containing high concentrations of polyaromatic hydrocarbons, polychlorinated biphenyls, and pesticides [41]. The effects of PGRs are likewise too. The ancillary enzyme GR activity significantly increased in the brain, heart, and liver but decreased in the erythrocyte and kidney of rats treated with both doses of PGRs. The GR enzyme has an important role in cellular antioxidant protection because it catalyzes the regeneration of GSH from GSSG. Its induced activity after 25 days of exposure reveals an enhanced production of GSSG predominantly in the erythrocyte and kidney of rats treated with both doses of PGRs. The depletion of GSH in the tissues supports this claim. However, it is not a general rule that when GSH-depleted GR activity always goes up due to a lot of factors participating in the synthesis. In general, it is demonstrated that fish from polluted sites have high GR activity because of higher peroxidative components in the polluted aquatic site. The drug-metabolizing enzyme GST activity significantly increased in the heart and kidney but decreased in the brain and erythrocytes of rats treated with both doses of PGRs. The decreased activity of GST may lead to decreased protection against oxidants [42]. However, the increased activities of GST are known to serve as protective responses to eliminate xenobiotics [43]. It is not a general rule that increases in pollutant concentrations induce antioxidant activity. Doyotte et al. [39] pointed out that a decreased response may accompany a first exposure to pollutants, which can be followed by an induction of antioxidant systems. Thus, the existence of an inducible antioxidant system may reflect an adaptation of organisms. Nevertheless, the physiological roles of these antioxidant enzymes in the cell are poorly understood because of complex interactions and interrelationships among individual components. Findings of this study suggest that further experiments should be performed to elicit what is responsible for the decreasing or increasing level of antioxidant defense systems, serum marker enzymes, and MDA. In addition, findings of this study suggest that the fluctuated levels in the constituents of antioxidant defense systems, serum marker enzymes and MDA content in the tissues of rats exposed to PGRs may be dependent on the differences between interstitial concentrations of PGRs. Namely, the systems might have to be exposed to different xenobiotic concentration due to blood volume differences in the tissues.

In conclusion, these results suggest that the serum marker enzymes, tissues antioxidant markers, and MDA content may offer means for monitoring toxicity of compounds such as PGRs. The test results may be used in oncoming investigations if more studies confirm our findings. Such a test will be of value in pollution studies and also be of interest to understand the molecular basis of refractoriness of PGRs toxicity.

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