Effects of Subacute and Subchronic Treatment of Synthetic Plant Growth Regulators on Liver Damage Serum Biomarkers Tissue Antioxidant Defense Systems and Lipid Peroxidation in Rats

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Abstract

The present study was aimed to investigate the effects of some synthetic plant growth regulators; ß-Naphthoxy acetic acid (ß-NOA) and 4-Chlorophenoxy acetic acid (4-CPA) at 10 and 20 ppm dosages on liver damage, antioxidant defense system and lipid peroxidation in rats. Rats were divided into 12 groups as control, ß-NOA and 4-CPA at 10 and 20 ppm dosages application groups. Serum marker enzymes and biochemical parameters were selected as biomarkers for liver damage. Antioxidant defense systems as SOD, GR, CAT, GSH-Px, GST and G6PD enzyme activities and GSH and MDA contents were determined in tissues as the indicators of oxidative stress. At the end of experiments, the statistically significant changes were observed in the levels of TPRO, TALB, TCHOL, TBIL, AST, ALT and LDH as liver damage serum biomarkers in rats exposed to 10 and 20 ppm of ß-NOA and 4-CPA as compared with controls. MDA levels increased in all the tissues of rats exposed to ß-NOA and 4-CPA dosages whereas the fluctuation in SOD, GR, CAT, GSH-Px, GST and G6PD enzyme activities and GSH levels were observed compared with controls. It could be concluded that the treatments of ß-NOA and 4-CPA dosages may induce liver damage and oxidative stress.

Keywords: ß-Naphthoxy acetic acid; 4-Chlorophenoxy acetic acid; Liver damage serum biomarkers; Antioxidant defense systems; Malondialdehyde; Rat

Abbreviations: ß-NOA: ß-Naphthoxy Acetic Acid; 4-CPA: 4-Chlorophenoxy Acetic Acid; AST: Aspartate Aminotransferase; ALT: Alanin Aminotransferase; LDH: Lactate Dehydrogenase; TPRO: Total Protein; TALB: Total Albumin; TCHOL: Total Cholesterol; TBIL: Total Bilirubin; SOD: Superoxide Dismutase; GR: Glutathione Reductase; CAT: Catalase; GSH-Px: Glutathione Peroxidase; GST: Glutathione-S-transferase; G6PD: Glucose-6-Phosphate Dehydrogenase; GSH: Glutathione; MDA: Malondialdehyde

Introduction

Many chemicals are currently used in agriculture, and plant growth regulators (PGRs) are among those widely used. The amounts of these substances placed into the environment may soon exceed those of insecticides [1]. PGRs play important roles in many cellular processes including seed development, dormancy, germination, vegetative growth, and environmental stress responses [2]. PGRs play also, important roles in many cellular processes including promotes stem elongation, overcomes dormancy in seed and buds, involved in parthenocarpic fruit development, flowering, mobilization of food reserves in grass seed germination, juvenility and sex expression [3].

Phenoxides mimicking the action of auxins, hormones chemically related to indole phosphoisic acid that stimulate growth in plants, have been used as plant growth regulators to increase the food production for more than 40 years [4]. 2,4-Dichlorophenoxyacetice acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 4-chlorophenoxyacetic acid (4-CPA) are the chemicals belonging to this group. Among these agents, teratogenic, mutagenic, and carcinogenic effects of 2,4-D and 2,4,5-T are subjected to intense investigations [5].

Free radicals, such as superoxide, hydroxyl ions and nitric oxide all contain an unpaired electron. These radicals can have a negative effect on cells causing oxidative damage that leads to cell death [6]. Antioxidant defenses, present in all aerobic organisms, include antioxidant enzymes and free-radical scavengers whose function is to remove reactive oxygen species, thus protecting whose function organisms from oxidative stress [7]. The sensitivity of cell to oxidants is attenuated by antioxidant defense system such as GSH, GST, CAT, SOD, GSH-Px, GR and glucose-6-phosphate dehydrogenase (G6PD). Among these enzymes, the GSH-Px, through reduction of both hydrogen peroxide and organic hydroperoxides, provide an efficient protection against oxidative damage and free radicals in the presence of GSH. Oxidized glutathione (GSSG) is regenerated by GR. SOD catalyses dismutation of superoxide anion radicals, whereas CAT eliminates hydrogen peroxide. Another group of enzymes, GST act as catalyst of 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 [8]. Oxidative stress may produce DNA damage, enzymatic inactivation, and peroxidation of cell constituents, especially lipid peroxidation when antioxidant defenses are impaired or overcome [9].

As a result of the industrial usage, this agrochemical is consumed by non-target-organisms [10]. Although PGRs are used for pest control and giving rise to the product on a wide variety of crops, little is known about the biochemical or physiological effects in mammalian organisms. However, there are some studies about endogenous PGRs including IBA. Furukawa et al. [11] indicated that IAA (indole acetic

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Received January 08, 2012; Accepted April 21, 2012; Published April 23, 2012

Citation: Ozok N, Celik I (2012) Effects of Subacute and Subchronic Treatment of Synthetic Plant Growth Regulators on Liver Damage Serum Biomarkers Tissue Antioxidant Defense Systems and Lipid Peroxidation in Rats. J Drug Metab Toxicol 3:124. doi:10.4172/2157-7609.1000124

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acids) might induce the neuronal apoptosis in the S phase and lead to microencephaly. de Melo et al. [12] determined that incubation for 24 h in the presence of IAA (1 mM) showed increase in the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase in rat neutrophils and lymphocytes. John et al. [13] observed that IAA possesses teratogenic effects in gestation mice and rats at 500 mg/kg/day. El-Moffy and Sakr [14] found that GA, induced liver neoplasms in Egyptian toads, and they suggested that the tumors could be diagnosed as hepatocellular carcinomas. GA, also induces microabscesses and hydropic degeneration in the liver and mononuclear inflammatory infiltration in the kidneys of laboratory mice, but not tumours. In a study, IAA effect investigated on human serum enzymes in vitro, it was found that IAA inhibited aspartate aminotransferase (AST) but activated amylase, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). Also, it was reported that while the levels of LDH and CPK increased significantly by IBA, the levels of AST, LDH and CPK were increased significantly by IAA after subacute exposure with 100 ppm dosages [15]. IAA was found to be linear-mixed type inhibitor for human serum BChE, and competitive inhibitor for the horse serum BChE enzyme [10]. Further, 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 [16-23]. On the other hand, a previous study carried out in the tissues of rats indicated that PGRs had neurotoxic and immunotoxic effect by deranging acetylcholinesterase, butyrylcholinesterase, adenosine deaminase and myeloperoxidase enzyme activities [24].

Despite the reasons mentioned in above paragraphs, little is known regarding the hepatotoxicity and oxidative stress effects of β-NOA and 4-CPA on vertebrate. In order to achieve a more rational design of β-NOA and 4-CPA, it is necessary to clarify the mechanism of hepatotoxicity and oxidative stress for β-NOA and 4-CPA. To this end, the treatments of β-NOA and 4-CPA were done orally because the effect of chemicals represents a well characterized in vivo toxicity model system. The parameters were chosen due to their important role for hepatotoxicity, oxidative stress damages and important role during detoxification in degradation and bioactivation of β-NOA and 4-CPA index. As it is known, liver damage serum parameters and oxidative stress biomarkers are sensitive to pollutants. Therefore, it is commonly used for ecotoxicological risk assessment and environmental pollutants monitoring studies. This study was approved by The Ethnic Committee of Yüzüncü Yıl University. Further, the research was supported by the University Grant Commission of Yüzüncü Yıl University.

Materials and Methods

Materials

PGRs, Thiobarbituric acid (TBA), Butylated hydroxytoluene (BHT), Trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), Reduced glutathione (GSH), Metaphosphoric acid, 5,5′dithiobis-(2-nitrobenzoic acid) (DTNB), Trihydroxymethyl aminomethane (Tris), 1-chloro-2, 4-dinitrobenzene (CDNB), β-Nicotinamide adenine dinucleotide phosphate (reduced) (NADPH), Oxidized glutathione (GSSG), Tris (2-KHz frequency ultrasonic, Jencons Scientific Co.) and Sodium chloride (NaCl) of technical grade used in this study. The tissues were homogenized for 5 min in 10 mL of 1 N NaOH and absolute ethanol, and then were stored at 4°C for 15 min. Then the pellets were centrifuged at 4000 g for 15 min at 4°C and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were used. The tissues were homogenized for 5 min in 10 mL of 1 N NaOH and absolute ethanol, and then were stored at 4°C for 15 min. Then the pellets were centrifuged at 4000 g for 15 min at 4°C and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were used. The tissues were homogenized for 5 min in 10 mL of 1 N NaOH and absolute ethanol, and then were stored at 4°C for 15 min. Then the pellets were centrifuged at 4000 g for 15 min at 4°C and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were used. The tissues were homogenized for 5 min in 10 mL of 1 N NaOH and absolute ethanol, and then were stored at 4°C for 15 min. Then the pellets were centrifuged at 4000 g for 15 min at 4°C and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were used. The tissues were homogenized for 5 min in 10 mL of 1 N NaOH and absolute ethanol, and then were stored at 4°C for 15 min. Then the pellets were centrifuged at 4000 g for 15 min at 4°C and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were used. The tissues were homogenized for 5 min in 10 mL of 1 N NaOH and absolute ethanol, and then were stored at 4°C for 15 min. Then the pellets were centrifuged at 4000 g for 15 min at 4°C and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were used. The tissues were homogenized for 5 min in 10 mL of 1 N NaOH and absolute ethanol, and then were stored at 4°C for 15 min. Then the pellets were centrifuged at 4000 g for 15 min at 4°C and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were used.
determined using the method described by Beutler et al [27]. GST 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 Beutler E [28]. GR activity was assayed according to Mannervik and Guthenberg [29] as the decrease in absorbance of NADPH at 340 nm. GSH-Px activity was assayed according to Paglia and Valentine [30] based on that of GSH-Px catalyses the oxidation of GSH by Cumene Hydroperoxide. SOD activity was measured at 505 nm and 37°C and calculated using inhibition percentage of formazan dye formation [31].

**Measurement of serum biomarkers**

AST, ALT and LDH serum enzyme levels, and biochemical parameters; TPRO, TALB, TCHOL TBIL levels were measured by an auto analyzer (BM/HITACHI-911), using the kits.

**Analysis of data**

All data were expressed as mean ± standard deviation (SD). The statistical analyses were made using the Minitab 13 for windows packet program. Means and Standard deviations were calculated according to the standard methods for all parameters. One way ANOVA statistical test was used to determine the differences between means of the treatments and the control group accepting the significance level at p ≤ 0.05.

**Results**

The results of experiment showed that the treatment of rats with ß-NOA and 4-CPA caused changes in the activities and levels of serum parameters selected as biomarkers for liver damage (Table 1-2), in the concentration of MDA and GSH, and antioxidant enzymes such as CAT, G6DP, GST, GR, GSH-Px and SOD in erythrocyte, liver, brain, kidney and heart tissues in comparison to control rats (Table 3-12). To find out the significance of biochemical changes in different tissues exposed to ß-NOA and 4-CPA for 25 and 50 days, the data have been subjected to ANOVA (One way) test. According to the results, ß-NOA and 4-CPA caused a significant fluctuate in serum biomarkers for liver damage with both periods. Also, the lipid peroxidation end product MDA significantly increased in the erythrocyte, liver, brain, kidney and heart tissues of rats treated with both the period of ß-NOA and 4-CPA. The GSH levels and antioxidant defense system enzymes

| Period | Parameters | Control X ± SD | ß-NOA 10 ppm X ± SD | ß-NOA 20 ppm X ± SD |
|--------|------------|----------------|---------------------|---------------------|
| SUBACUTE | AST (U/L) | 113.5 ± 3.7 | 134.5 ± 12.3* | 176.5 ± 17.6* |
| ALT (U/L) | 56.1 ± 3.3 | 61.1 ± 5.2 | 62.2 ± 3.3* |
| LDH (U/L) | 697.8 ± 9.5 | 908.7 ± 15.7* | 989.4 ± 16.6* |
| TP (g/dL) | 6.3 ± 0.2 | 6.5 ± 0.8 | 7.129 ± 0.8 |
| GSH-Px (mg/dL) | 3.8 ± 0.07 | 4.3 ± 0.4 | 4.0 ± 0.2 |
| TAIL (g/dL) | 43.1 ± 3.2 | 43.5 ± 7.1 | 59.7 ± 6.4* |
| TBL (mg/dL) | 0.06 ± 0.011 | 0.08 ± 0.004* | 0.08 ± 0.009* |

Each value represents the Mean ± SD. * p<0.05

**Table 1**: Effects of ß-NOA 10-20 ppm on serum biomarker levels of rats at subacute and subchronic.

| Period | Parameters | Control X ± SD | 4-CPA 10 ppm X ± SD | 4-CPA 20 ppm X ± SD |
|--------|------------|----------------|---------------------|---------------------|
| SUBACUTE | AST (U/L) | 118.1 ± 8.4 | 191.4 ± 5.6* | 187.4 ± 15.6* |
| ALT (U/L) | 46.1 ± 4.0 | 62.1 ± 4.2* | 53.8 ± 4.0* |
| LDH (U/L) | 595.4 ± 11.0 | 824.7 ± 24.4* | 1012.2 ± 23.8* |
| TP (g/dL) | 6.6 ± 0.6 | 7.1 ± 0.5 | 6.5 ± 0.4 |
| GSH-Px (mg/dL) | 4.7 ± 0.4 | 4.2 ± 0.2 | 3.9 ± 0.3* |
| TAIL (mg/dL) | 46.4 ± 4.6 | 48.5 ± 5.6 | 53.2 ± 5.0 |
| TBL (mg/dL) | 0.06 ± 0.009 | 0.07 ± 0.011 | 0.08 ± 0.01* |

Each value represents the Mean ± SD. * p<0.05

**Table 2**: Effects of 4-CPA 10-20 ppm on serum biomarker levels of rats at subacute and subchronic.
Discussion

In recent years, a significant increase in the use of PGRs against harmful agricultural pests and giving rise to losing product have been observed in Turkey and 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 this study, ß-NOA and 4-CPA were preferred because information on its negative effects on higher animals is very limited for in vivo, oral exposures. Also, ß-NOA and 4-CPA are found in plants as exogen hormones and 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 to ß-NOA and 4-CPA caused changes in the activities and levels of serum parameters selected as biomarkers for liver damage, the production of lipid peroxides, and affected antioxidant defense in various rat tissues.

So far, no study examining the effect of ß-NOA and 4-CPA in vivo have been made on rat erythrocyte and that of tissues MDA content and antioxidant enzymes activities. Therefore, we could not have the chance to compare our results with the previous results. In addition, because of high variability in analyzing MDA content and antioxidant enzymes-chemicals interaction in vitro and in vivo, and inconsistent factors like treatment time and manner, the setting of studies, purity of chemicals and species tissue differences etc., it is difficult to compare the present data to different studies regarding the toxicological effect.

To the extent that chemical affect, little is known about the biochemical or physiological effects in vertebrates. Ozdem et al. [5] observed that 4-CPA-raised tomato homogenate fluctuate the rat erythrocyte antioxidant enzymes such as G6PD, CAT, selenium-dependent GSH-Px and Cu/Zn-SOD.

In this study, ß-NOA and 4-CPA caused a significant alteration in the activities and levels of serum parameters selected as TPRO, TALB,
TCHOL, TBIL, AST, ALT and LDH biomarkers for liver damage (Tables 1-2). Namely, ß-NOA and 4-CPA caused a significant fluctuate the level serum biomarker for hepatotoxicity with both periods and dosages. The reasons for such effect of alcohol and the grape seeds supplementation are not understood at present certainly. However, it is known that several soluble enzymes in blood serum such as these enzymes have been considered as indicators of the hepatic dysfunction and damage. Also, the increase in the activities of AST and ALT in plasma of rats treated with ethyl alcohol is mainly due to the leakage of these enzymes from the liver cytosol into the bloodstream [32]. Further, ALT and AST levels are also of value indicating the existence of liver diseases, as this enzyme is present in large quantities in the liver. ALT increases in serum when cellular degeneration or destruction occurs in this organ [33]. Any interference in these enzymes leads to biochemical impairment and lesions of the tissue and cellular function Yousef et al. [35] reported that the changes in the activities of these enzymes in SnCl₂-treated rats were regarded as the biochemical manifestation of the toxic action of inorganic tin. On the other hand, phosphatases and dehydrogenases are important and critical enzymes in biological processes too. They are responsible for detoxification, metabolism and biosynthesis of energetic macromolecules for different essential functions. The increase in plasma LDH activity may be due to the hepatocellular necrosis leading to leakage of the enzyme to the blood stream [36]. Thus, when alcohol may lead to the release of these enzymes into plasma as a result of autolytic breakdown or cellular necrosis, the grape seeds supplement impart protection against alcohol induced oxidative injury that may result in development of liver damage. Similarly, another researcher had determined that have decreased AST and ALT activities in the serum of Channa striatus following exposure to xenobiotics [37]. Oruc and Uner [38] also found an increase in the serum LDH activity in Cyprinus carpio following exposure to 2,4-Diamin. Although the treatment, materials of studies and the setting of studies are different, this result is in accordance with our result partly.

### Table 5: Effects of ß-NOA 10-20 ppm on antioxidant defence systems and MDA contents in heart of rats at subacute and subchronic.

| Period  | Parameters | Control X ± SD | ß-NOA 10 ppm X ± SD | ß-NOA 20 ppm X ± SD |
|---------|------------|----------------|---------------------|---------------------|
|         | SOD U/g    | 2258.4 ± 12.7  | 2242.6 ± 13.5       | 2225.2 ± 26.5       |
|         | GR U/g     | 0.6 ± 0.1      | 0.7 ± 0.04          | 0.8 ± 0.09          |
|         | CAT U/g    | 36.8 ± 4.7     | 29.5 ± 4.9          | 27.6 ± 5.3          |
|         | GSH-Px U/g | 64.2 ± 1.9     | 52.3 ± 1.4          | 44.7 ± 2.1          |
|         | GST U/g    | 3.4 ± 0.2      | 2.5 ± 0.3           | 3.6 ± 0.2           |
|         | G6PD U/g   | 0.3 ± 0.02     | 0.2 ± 0.03          | 0.1 ± 0.06          |
|         | GSH mg/g   | 19.6 ± 0.4     | 16.7 ± 1.2          | 12.0 ± 0.7          |
|         | MDA nmol/g | 2.2 ± 0.3      | 6.0 ± 0.6           | 24.9 ± 0.9          |
| **SUBACUTE** |            |                |                      |                     |
|         | SOD U/g    | 2274.4 ± 13.1  | 2247.7 ± 44.5       | 2259.0 ± 12.9       |
|         | GR U/g     | 0.6 ± 0.02     | 0.3 ± 0.05          | 0.3 ± 0.05          |
|         | CAT U/g    | 33.5 ± 5.1     | 25.0 ± 3.9          | 21.7 ± 2.8          |
|         | GSH-Px U/g | 94.1 ± 4.3     | 83.1 ± 3.4          | 66.3 ± 2.4          |
|         | GST U/g    | 3.4 ± 0.1      | 3.0 ± 0.2           | 2.9 ± 0.1           |
|         | G6PD U/g   | 0.39 ± 0.03    | 0.35 ± 0.05         | 0.33 ± 0.02         |
|         | GSH mg/g   | 20.3 ± 0.4     | 16.4 ± 0.7          | 13.8 ± 0.2          |
|         | MDA nmol/g | 2.4 ± 0.7      | 15.1 ± 1.1          | 14.7 ± 1.3          |
| **SUBCHRONIC** |        |                |                      |                     |
|         | SOD U/g    | 2191.4 ± 33.0  | 2151.3 ± 23.5       | 2191.1 ± 32.2       |
|         | GR U/g     | 0.9 ± 0.07     | 0.8 ± 0.08          | 0.9 ± 0.07          |
|         | CAT U/g    | 39.7 ± 6.5     | 13.0 ± 2.5          | 12.8 ± 3.0          |
|         | GSH-Px U/g | 65.9 ± 2.8     | 52.3 ± 4.0          | 47.7 ± 2.9          |
|         | GST U/g    | 3.2 ± 0.1      | 4.4 ± 0.3           | 3.5 ± 0.2           |
|         | G6PD U/g   | 0.3 ± 0.02     | 0.2 ± 0.03          | 0.1 ± 0.03          |
|         | GSH mg/g   | 17.4 ± 0.7     | 17.3 ± 0.2          | 11.3 ± 1.3          |
|         | MDA nmol/g | 2.0 ± 0.5      | 24.0 ± 1.2          | 25.5 ± 1.1          |

Each value represents the Mean ± SD. * p<0.05

### Table 6: Effects of 4-CPA 10-20 ppm on antioxidant defence systems and MDA contents in heart of rats at subacute and subchronic.

| Period  | Parameters | Control X ± SD | 4-CPA 10 ppm X ± SD | 4-CPA 20 ppm X ± SD |
|---------|------------|----------------|---------------------|---------------------|
|         | SOD U/g    | 2191.4 ± 33.0  | 2151.3 ± 23.5       | 2191.1 ± 32.2       |
|         | GR U/g     | 0.9 ± 0.07     | 0.8 ± 0.08          | 0.9 ± 0.07          |
|         | CAT U/g    | 39.7 ± 6.5     | 13.0 ± 2.5          | 12.8 ± 3.0          |
|         | GSH-Px U/g | 65.9 ± 2.8     | 52.3 ± 4.0          | 47.7 ± 2.9          |
|         | GST U/g    | 3.2 ± 0.1      | 4.4 ± 0.3           | 3.5 ± 0.2           |
|         | G6PD U/g   | 0.3 ± 0.02     | 0.2 ± 0.03          | 0.1 ± 0.03          |
|         | GSH mg/g   | 17.4 ± 0.7     | 17.3 ± 0.2          | 11.3 ± 1.3          |
|         | MDA nmol/g | 2.0 ± 0.5      | 24.0 ± 1.2          | 25.5 ± 1.1          |
| **SUBACUTE** |        |                |                      |                     |
|         | SOD U/g    | 2272.8 ± 20.6  | 2266.1 ± 5.1        | 2247.9 ± 16.8       |
|         | GR U/g     | 1.0 ± 0.05     | 0.3 ± 0.04          | 0.2 ± 0.02          |
|         | CAT U/g    | 25.8 ± 2.3     | 24.8 ± 2.0          | 24.5 ± 3.7          |
|         | GSH-Px U/g | 107.2 ± 3.7    | 108.1 ± 3.6         | 93.0 ± 4.5          |
|         | GST U/g    | 3.4 ± 0.1      | 1.9 ± 0.6           | 3.1 ± 0.7           |
|         | G6PD U/g   | 0.3 ± 0.02     | 0.3 ± 0.03          | 0.3 ± 0.03          |
|         | GSH mg/g   | 15.0 ± 1.0     | 7.5 ± 1.1           | 3.2 ± 1.2           |
|         | MDA nmol/g | 12.4 ± 1.8     | 18.9 ± 1.7          | 23.6 ± 1.8          |

Each value represents the Mean ± SD. * p<0.05

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In addition to the fluctuated serum marker parameters, the results of the present study have also demonstrated that the rats treated with both doses of ß-NOA and 4-CPA could have affected the antioxidant defense systems in vertebrates. This is evidenced from our observation that, upon ß-NOA and 4-CPA treatment in vivo, the concentration of MDA and the antioxidant defense markers in erythrocyte, liver, brain, kidney and heart tissues differ from that of control rats. The present study showed that the lipid peroxidation end product MDA significantly increased in the all tissues of rats treated with both the period and dosages of ß-NOA and 4-CPA. The reasons for such affect of PGRs are not understood at the present. But, the increased content of MDA may result from an increase of hydroxyl radicals (.OH). However, it is conceivable that ß-NOA and 4-CPA might be interacting primarily with the tissues, resulting in lipid peroxidation processes by way of increase superoxide radicals as 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 [39]. It is known that OH can initiate lipid peroxidation in tissues [8]. Results also showed that the GSH levels were significantly fluctuated in the tissues of rats treated with both the period and dosages of ß-NOA and 4-CPA. On the other hand, it is known that the elevation of lipid peroxidation after the consumption of some xenobiotics and following superoxide overproduction which produce dismutation singlet oxygen and H2O2, can be easily converted later into the reactive .OH. Both single oxygen and OH radical have a high potential to initiate free radicals chain reactions of lipid peroxidation. Further, it is known that OH can initiate lipid peroxidation in tissues [40] and MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation [39].

Meanwhile, SOD, GR, GSH-Px and GST activities and GSH levels were fluctuated at appreciable level in the alcohol-treated rats. But the efficacy of the grape seeds against these fluctuations could have

| Period | Parameters | Control X ± SD | ß-NOA 10 ppm X ± SD | ß-NOA 20 ppm X ± SD |
|--------|------------|---------------|---------------------|---------------------|
| SUBACUTE | SOD U/g | 2272.6 ± 23.3 | 2169.8 ± 21.4* | 2239.5 ± 27.7 |
| | GR U/g | 20.0 ± 0.1 | 1.9 ± 0.1 | 2.0 ± 0.1 |
| | CAT U/g | 202.9 ± 18.1 | 196.1 ± 18.1 | 198.3 ± 8.8 |
| | GSH-Px U/g | 243.6 ± 9.7 | 210.8 ± 7.0* | 207.4 ± 8.6 |
| | GST U/g | 2.4 ± 0.2 | 2.1 ± 0.2 | 3.0 ± 0.3 |
| | G6PD U/g | 1.0 ± 0.08 | 1.0 ± 0.09 | 1.1 ± 0.09 |
| | GSH mg/g | 8.4 ± 0.7 | 5.5 ± 0.7* | 5.0 ± 0.8* |
| | MDA nmol/g | 39.5 ± 1.0 | 76.6 ± 4.2' | 79.4 ± 3.9' |
| SUBCHRONIC | SOD U/g | 2329.0 ± 1.7 | 2233.6 ± 19.9* | 2238.0 ± 15.9' |
| | GR U/g | 2.2 ± 0.2 | 1.8 ± 0.2' | 1.4 ± 0.1' |
| | CAT U/g | 198.2 ± 16.2 | 219.3 ± 17.2 | 195.6 ± 21.8 |
| | GSH-Px U/g | 222.4 ± 8.1 | 186.9 ± 8.6' | 189.0 ± 7.1' |
| | GST U/g | 2.3 ± 0.1 | 1.7 ± 0.2 | 1.7 ± 0.1' |
| | G6PD U/g | 1.1 ± 0.09 | 1.0 ± 0.08 | 1.0 ± 0.06 |
| | GSH mg/g | 7.2 ± 0.3 | 6.0 ± 0.6' | 6.2 ± 0.6' |
| | MDA nmol/g | 40.0 ± 3.0 | 54.1 ± 3.4' | 64.4 ± 3.0' |

Each value represents the Mean ± SD, * p<0.05

| Period | Parameters | Control X ± SD | 4-CPA 10 ppm X ± SD | 4-CPA 20 ppm X ± SD |
|--------|------------|---------------|---------------------|---------------------|
| SUBACUTE | SOD U/g | 2170.9 ± 17.0 | 2170.1 ± 13.5 | 2148.5 ± 15.2' |
| | GR U/g | 2.0 ± 0.1 | 1.8 ± 0.08' | 1.5 ± 0.1' |
| | CAT U/g | 191.5 ± 15.3 | 135.2 ± 17.1 | 70.7 ± 11.4' |
| | GSH-Px U/g | 276.8 ± 9.6 | 200.9 ± 8.8' | 172.6 ± 9.7 |
| | GST U/g | 3.3 ± 0.2 | 3.1 ± 0.2 | 3.3 ± 0.1 |
| | G6PD U/g | 1.0 ± 0.08 | 1.1 ± 0.06' | 0.9 ± 0.08 |
| | GSH mg/g | 4.8 ± 0.4 | 6.4 ± 0.6' | 3.5 ± 0.2' |
| | MDA nmol/g | 38.5 ± 0.6 | 44.2 ± 1.4' | 46.8 ± 3.8' |
| SUBCHRONIC | SOD U/g | 2229.4 ± 15.5 | 2217.5 ± 18.4 | 2222.9 ± 16.9 |
| | GR U/g | 2.4 ± 0.2 | 2.6 ± 0.4 | 2.5 ± 0.1 |
| | CAT U/g | 189.0 ± 16.1 | 140.1 ± 8.0' | 153.5 ± 9.5 |
| | GSH-Px U/g | 204.8 ± 7.7 | 178.0 ± 9.5' | 174.7 ± 8.5' |
| | GST U/g | 3.4 ± 0.2 | 3.1 ± 0.3 | 3.1 ± 0.2 |
| | G6PD U/g | 1.0 ± 0.04 | 1.1 ± 0.01' | 1.1 ± 0.02' |
| | GSH mg/g | 4.5 ± 0.5 | 5.9 ± 0.4' | 6.3 ± 0.6' |
| | MDA nmol/g | 32.5 ± 0.8 | 49.4 ± 0.3' | 53.0 ± 2.4' |

Each value represents the Mean ± SD, * p<0.05

Table 7: Effects of ß-NOA 10-20 ppm on antioxidant defence systems and MDA contents in kidney of rats at subacute and subchronic.

Table 8: Effects of 4-CPA 10-20 ppm on antioxidant defence systems and MDA contents in kidney of rats at subacute and subchronic.
The reasons for such effect of functional plant’s supplemented are not understood at the present. However, oxidative stress can affect the activities of protective enzymatic antioxidants in organisms exposed to alcohol. The increased GSH-Px and GST activities may reflect an adaptive change against ethanol-induced lipid peroxide toxicity [41]. However, the increased activities of GST are known to serve as protective responses to eliminate xenobiotics [42]. Thus, the existence of an inducible antioxidant system may reflect an adaptation of organisms. An increase in the constituent of antioxidant defense systems may result an increase of superoxide radicals. Further, the decreased activity of GST may lead to decreased protection against oxidants [43]. It is not a general rule that increases in pollutant concentrations induce antioxidant activity. Doyotte et al. [44] 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 role of a single antioxidant enzyme in the cell is 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 elevation of MDA content in tissues, and for the decreasing or increasing level of antioxidant enzymes. In addition, the different values of antioxidants marker enzymes and MDA content in the tissues of rats exposed to PGRs may dependent on differences of interstitial. Namely, the systems might have to be exposed to different xenobiotic concentration due to blood volume differences in the tissues. 

Enzymatic antioxidants such as SOD, GR, GSH-Px, 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 [45]. The increased activities of SOD, CAT, GSH-Px, GR, and GST are known to serve as protective responses to eliminate xenobiotics [46]. Each value represents the Mean ± SD. * p<0.05

| Parameters      | Control             | 8-NOA 10 ppm       | 8-NOA 20 ppm       |
|-----------------|---------------------|--------------------|--------------------|
|                 | X ± SD              | X ± SD             | X ± SD             |
| SOD U/g         | 2217.6 ± 14.7       | 2215.3 ± 23.2      | 2212.1 ± 27.2      |
| GR U/g          | 1.2 ± 0.07          | 1.1 ± 0.07         | 1.0 ± 0.08         |
| CAT U/g         | 319.9 ± 15.4        | 333.1 ± 14.5       | 313.1 ± 12.5       |
| GSH-Px U/g      | 54.9 ± 1.9          | 25.0 ± 2.4         | 23.7 ± 2.0         |
| GST U/g         | 8.5 ± 0.3           | 6.9 ± 0.8          | 8.1 ± 0.4          |
| G6PD U/g        | 2.8 ± 0.2           | 1.2 ± 0.1          | 0.6 ± 0.1          |
| GSH mg/g        | 25.6 ± 0.4          | 25.6 ± 0.2         | 24.8 ± 0.5         |
| MDA nmol/g      | 14.8 ± 1.5          | 20.1 ± 1.6         | 26.5 ± 1.0         |

| Period          | Parameters | Control             | 4-CPA 10 ppm       | 4-CPA 20 ppm       |
|-----------------|------------|---------------------|--------------------|--------------------|
|                 | X ± SD     | X ± SD              | X ± SD             | X ± SD             |
| SOD U/g         | 2270.5 ± 15.6       | 2266.4 ± 20.4       | 2242.9 ± 12.8      |
| GR U/g          | 1.0 ± 0.09    | 0.8 ± 0.08          | 0.6 ± 0.08         |
| CAT U/g         | 315.8 ± 7.1    | 237.7 ± 1.4         | 299.9 ± 5.7        |
| GSH-Px U/g      | 71.9 ± 2.8    | 53.0 ± 4.2          | 53.7 ± 1.9         |
| GST U/g         | 9.2 ± 0.1     | 8.0 ± 0.7           | 9.4 ± 0.4          |
| G6PD U/g        | 2.7 ± 0.3     | 3.5 ± 0.2           | 2.7 ± 0.3          |
| GSH mg/g        | 23.0 ± 0.3    | 15.8 ± 0.8          | 13.8 ± 0.5         |
| MDA nmol/g      | 16.9 ± 0.8    | 53.1 ± 1.8          | 61.6 ± 3.4         |

| Period          | Parameters | Control             | 4-CPA 10 ppm       | 4-CPA 20 ppm       |
|-----------------|------------|---------------------|--------------------|--------------------|
|                 | X ± SD     | X ± SD              | X ± SD             | X ± SD             |
| SOD U/g         | 2206.6 ± 17.2       | 2199.9 ± 11.5       | 2194.1 ± 20.9      |
| GR U/g          | 1.0 ± 0.04    | 1.1 ± 0.1           | 1.0 ± 0.1          |
| CAT U/g         | 367.7 ± 22.8   | 353.2 ± 30.5        | 327.0 ± 32.0       |
| GSH-Px U/g      | 45.0 ± 2.9    | 26.4 ± 2.5          | 34.3 ± 2.7         |
| GST U/g         | 8.7 ± 0.4     | 8.0 ± 0.9           | 9.5 ± 0.3          |
| G6PD U/g        | 3.2 ± 0.4     | 2.5 ± 0.3           | 2.4 ± 0.2          |
| GSH mg/g        | 25.5 ± 0.7    | 25.7 ± 0.7          | 11.3 ± 1.8         |
| MDA nmol/g      | 9.1 ± 0.5     | 19.8 ± 1.8          | 30.6 ± 3.4         |

| Period          | Parameters | Control             | 4-CPA 10 ppm       | 4-CPA 20 ppm       |
|-----------------|------------|---------------------|--------------------|--------------------|
|                 | X ± SD     | X ± SD              | X ± SD             | X ± SD             |
| SOD U/g         | 2260.7 ± 24.1       | 2256.1 ± 23.8       | 2251.0 ± 20.8      |
| GR U/g          | 1.1 ± 0.08    | 0.4 ± 0.05          | 0.3 ± 0.04         |
| CAT U/g         | 355.7 ± 17.5   | 341.4 ± 14.2        | 295.1 ± 29.4       |
| GSH-Px U/g      | 76.4 ± 2.4    | 72.1 ± 1.5          | 66.6 ± 2.1         |
| GST U/g         | 9.2 ± 0.1     | 9.1 ± 0.5           | 9.0 ± 0.4          |
| G6PD U/g        | 3.0 ± 0.1     | 2.6 ± 0.1           | 2.3 ± 0.1          |
| GSH mg/g        | 24.0 ± 0.7    | 28.1 ± 0.5          | 23.8 ± 0.4         |
| MDA nmol/g      | 11.8 ± 0.9    | 62.7 ± 3.8          | 66.6 ± 2.9         |
to eliminate reactive free radicals [46]. However, Candeias et al. [16] investigated the peroxidation of liposomes by a haem peroxidase and hydrogen in the presence of IAA and derivates. They found that these compounds can accelerate lipid peroxidation up to 65-fold, and this is attributed to the formation of peroxyl radicals that may react with the lipids, possibly by hydrogen abstraction.

**Conclusion**

The observations presented here led us to conclude that while administration of subacute and subchronic ß-NOA and 4-CPA at sub lethal dosages promotes MDA concentration fluctuations in the antioxidative systems and liver damage serum biomarkers. Also, these results suggest that the serum biomarkers, tissues antioxidant markers and MDA content may be offer with means for monitoring toxicity of compounds such as ß-NOA and 4-CPA. 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 molecular basis of refractoriness ß-NOA and 4-CPA toxicity.

**Acknowledgements**

None of the authors has a commercial interest, financial interest, and/or other relationship with manufacturers of pharmaceuticals, laboratory supplies, and/or medical devices or with commercial providers of medically related services. The authors are grateful to the University of Yuzuncu Yil Grant Commission for providing financial assistance during the tenure of research numbered with YYÜ-BAP-2010-FBE-D104. Ismail Celik was the main moderator of the study. Necati Ozok performed the biochemical investigation and treatment in this study.

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Citation: Ozok N, Celik I (2012) Effects of Subacute and Subchronic Treatment of Synthetic Plant Growth Regulators on Liver Damage Serum Biomarkers Tissue Antioxidant Defense Systems and Lipid Peroxidation in Rats. J Drug Metab Toxicol 3:124. doi:10.4172/2157-7609.1000124

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