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Abcisic acid and gibberellic acid cause increased lipid peroxidation and fluctuated antioxidant defense systems of various tissues in rats

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Abstract

The study was aimed at demonstrating changes in the antioxidant defense systems [Reduced glutathione (GSH), glutathione reductase (GR), superoxide dismutase (SOD), glutathione-S-transferase (GST) and catalase (CAT)] forming an antioxidative barrier and oxidative stress parameter (Malondialdehyde = MDA) in the various tissues of Sprague–Dawley rats which were administrated plant growth regulators (PGRs) [Abcisic acid (ABA) and Gibberellic acid (GA_3)] during 25 days. Seventy five parts per million of ABA and GA_3 as drinking water were administered orally to rats (Sprague–Dawley albino) ad libitum for 25 days continuously. The PGRs treatments caused different effects on antioxidant defense systems and MDA content of experimented rats compared to controls. The lipid peroxidation end product MDA significantly increased in the spleen and lungs of rats treated with ABA and GA_3 without significantly change in the other tissues. The GSH levels were significantly increased in the lungs and stomach of rats treated with ABA without any change in the tissues of rats treated with GA_3. Antioxidant enzyme activities such as SOD significantly increased in the spleen of rats treated with ABA and GA_3. Meanwhile, SOD significantly increased in the kidney of rats treated with GA. CAT significantly decreased in the lungs treated with ABA but did not change significantly in all the rest of rat tissues treated with both the PGRs. On the other hand, the ancillary enzyme GR activity decreased in the spleen and increased in the kidney with GA_3 treatment. The drug metabolizing enzyme GST activity significantly decreased in the heart of rats treated with GA_3 but increased in the spleen and lungs of rats treated with both PGRs.

The observations presented led us to conclude that administration of subacute ABA and GA_3 promotes lipid peroxidatin content and alters in the antioxidative systems in the rat's various tissues. These data, along with changes, suggest that the PGRs produced oxidative stress in rats during the period of a 25-day subchronic exposure.

Keywords: Plant growth regulators; Antioxidant enzymes; Malondialdehyde; Rat

1. Introduction

Many chemicals are currently used in agriculture, and PGRs are among those widely employed. The amounts of these substances placed into the environment may soon exceed those of insecticides [1]. ABA plays important roles in many cellular processes including seed development, dormancy, germination, vegetative growth, and environmental stress responses. These diverse functions of ABA involve complex regulatory mechanisms that control its production, degradation, signal perception, and transduction [2]. GA_3 also plays, important roles in many cellular processes including promoting stem elongation, overcoming dormancy in seed and buds, involved in parthenocarpic fruit development, flowering, mobilization of food reserves in grass seed germination, juvenility and sex expression [3].

Abbreviations: ADA, adenosine deaminase; ALT, alanin aminotransferase; AST, aspartate aminotransferase; BHT, butylated hydroxytoluene; CAT, catalase; CDNB, 5,5′dithiobis-(2-nitrobenzoic acid); CPK, creatine phosphokinase; EDTA, ethylenediaminetetraacetic acid; GPx, glutathione peroxidase; GSH, reduced glutathione; GR, glutathione reductase; G6PD, glucose-6-phosphate dehydrogenase; IAA, indole acetic acid; IBA, indole butyric acid; Kn, kinetin; LDH, lactate dehydrogenase; NADPH, β-nicotinamide adenine dinucleotide phosphate (reduced); MDA, malondialdehyde; MPO, myeloperoxidase; GSSG, oxidized glutathione; PGRs, plant growth regulators; Ppm, parts per million; SOD, superoxide dismutase

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Although PGRs are used for pest control and giving rise product on a wide variety of crops, little is known about the biochemical or physiological effects in mammalian organisms. There is increased oxidative stress obtained from a polluted area containing high concentrations of polyaromatic hydrocarbons, polychlorinated biphenyls, PGRs and pesticides. 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 [4]. However, little is known of how different PGRs classes contribute to the oxidative stress induced by these xenobiotics. In the literature, it is reported that fecundity, longevity and egg viability have been changed in different insects by PGRs treatment [5–9]. Furukawa et al. [10] indicated that indole acetic acid (IAA) might induce the neuronal apoptosis in the S phase and lead to microencephaly. Hsiao et al. [11] results' suggest that kinetin has effective free radical-scavenging activity in vitro and antithrombotic activity in vivo. Also, de Melo et al. [12] determined that incubation for 24 h in the presence of IAA (1 mM) showed increase in the activities of SOD, 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. Ozmen et al. [14] observed that ABA and GA_3 affect on sexual differentiation and some physiological parameters of laboratory mice. Also, it is reported that PGRs causes increase in the number of splenic plaque forming cells and circulating white blood cells, hematocrit values, and thymus weight in young deer mice [15]. El-Mofty and Sakr [16] found that GA_3 induced liver neoplasm in Egyptian toads, and they suggested that the tumours could be diagnosed as hepatocellular carcinomas. GA_3 also induces microabsceses and hydropic degeneration in the liver and mononuclear inflammatory infiltration in the kidneys of laboratory mice, but not tumours. On the other hand, some PGRs have been shown to affect the antioxidant defense systems and MDA content [17,18]. The effects of IAA and kinetin (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) [19]. Also, it was reported that while the levels of LDH and CPK were increased significantly by indole butyric acid (IBA), the levels of AST, LDH and CPK were increased significantly by IAA. In addition, the levels of AST, LDH and CPK were increased significantly by Kn [20]. 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 [21–24]. According to The U.S. Environmental Protection Agency (EPA), toxic xenobiotic chemicals are irritating to the eyes, skin and mucous membrane and since it is easily entered into organism such as orally or by inhalation and can injure liver, kidney, muscle and brain tissues.

In spite of reason above, there are still some unresolved issues about how PGRs affects on vertebrata. In order to achieve a more rational design of PGRs, it is necessary to clarify the mechanism of toxicity for PGRs tested and develop an understanding of structure–toxicity relationships. For this aim, the treatments of ABA and GA_3 were done orally because the effect of chemicals represents a well characterized in vivo toxicity model system. The tissues were chosen due to its important role during detoxification in degradation and bioactivation of the chemicals.

2. Materials and methods

2.1. 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), 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. (United Kingdom BT29 4QY).

2.2. Animals

Rats (Sprague–Dawley albino) 4 months of age with an average weighing 250–300 g were provided by the animal house of the Medical School of Yüzüncü Yıl University, and were housed in three 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 [25]. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments.

2.3. Treatment of rats

This investigation was performed on male rats. The animals are housed for a minimum of 5 days to 'acclimatize' before being dosed with the substance. The rats were exposed to 75 ppm ABA and GA_3 ad libitum for 25 days as drinking water. Seventy five milligrams of the ABA and GA_3 were dissolved in 1 ml of 1N NaOH, and then were diluted with tap water until 1000 ml to obtain a 75 ppm dose. For the control rats, only 1 ml of 1N NaOH was added to 1000 ml of tap water. Because the PGRs are photoactive compounds the drinking water containing ABA and GA_3 were prepared and refreshed every day in amber bottle. Since all rats have the same physiologic characters, daily water consumption of all groups rat was approximately 30 ± 3 ml during the tests. Consequently, the PGRs intake amount of each rat was about 2.2 ± 0.3 mg/day.

At the end of the treatments, the rats were anesthetized by inhalation of diethyl ether and the rat's lungs, spleen, stom-

ach, heart and kidney tissue samples were obtained. 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. For obtaining tissues supernatants; the tissues were homogenized for 5 min in 50 mM ice-cold KH_2PO_4 buffer solution (pH 7.0) (1:10, w/v; 0.5 g tissue + 5 ml buffer solution) using a glass-porcelain homogenizer (20 kHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000 × *g* for 15 min. All processes were carried out at 4 °C. Supernatants were used to determine antioxidant defense system constituents and MDA concentration.

2.4. Biochemical analysis

The tissues MDA concentration was determined using the method described by Jain et al. [26], based on TBA reactivity. Briefly, 0.2 ml 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 (4000 × *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 nm for tissues MDA concentration (Novaspec II Pharmacia-Biotech, Biochrom Ltd., UK).

The tissues GSH concentration was measured using the method described by Beutler et al. [27]. Briefly, 0.2 ml fresh supernatant was added to 1.8 ml distilled water. Three milliliters 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 milliliters 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 [28]. 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). 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 [29]. GR (EC 1.6.4.2) activity was assayed at 37 °C and 340 nm by following the oxidation of NADPH by GSSG [31]. SOD (EC 1.15.1.1) activity was measured at 505 nm and 37 °C and calculated using inhibition percentage of formazon formation [30].

2.5. Analysis of data

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

3. Results and discussion

In the present study, ABA and GA_3 were preferred because the information on their negative effects on higher animals is very limited for in vivo, oral exposures. Also, ABA and GA_3 are found in wide variety of biologically active compounds. The data collected in this study were from one time-point of the experiment. We found that the treatment to ABA and GA_3 affected the content of MDA and antioxidant defense systems in the various tissues of rats.

So far, no study examining the effect of ABA and GA_3 in vivo as drinking water has been made on MDA content and antioxidant defense of these tissues. Therefore, we could not have the chance to compare our results with the previous results. However, Tuluce and Celik [21] have investigated the effects of ABA and GA_3 on serum marker enzymes, antioxidant defense systems and MDA concentration in different tissues of rats. Also, Çelik et al. [24] have determined the effects of ABA and GA_3 on different antioxidant defense system and MDA content in these same tissues of rats for 50 days drinking water study. Furthermore, Celik and Tuluce [17] have searched the effects of IAA and Kn on different antioxidant defense system in these tissues of rats. On the other hand, de Melo et al. [12] found that the incubation of neutrophils and lymphocytes of rat for 24 h in the presence of IAA (1 mM) showed an increase in CAT activity. Ciftci et al. [31] also determined that both in vivo and in vitro treatment of spermidine and kinetin did not affect enzymatic activity of carbonic anhydrase (CA) and G6PD whereas putrescine decreased and ABA increased the activity of the enzymes. These studies are partly in agreement with our results although the different treatments, materials and the settings of experiments are different. In addition, because of high variability in analyzing MDA 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 and species tissue differences etc., it is difficult to compare the present data to previous studies regarding for the toxicological effect.

The results of the present study have demonstrated that ABA and GA_3 could have affected the antioxidant defense system and MDA content in vertebrates. This is evidenced from our observation that, upon ABA and GA_3 treatment in vivo, the activity of the enzymes and GSH levels in the spleen, lungs, stomach, heart and kidney differ from that of controls. ABA and GA_3 caused significant alterations in the level of enzymes in almost all the tissues (Figs. 1–4). According to the results, ABA and GA_3 caused a significant decrease in the activity of GR in the spleen whereas GA_3 caused an increase in GR activity in the

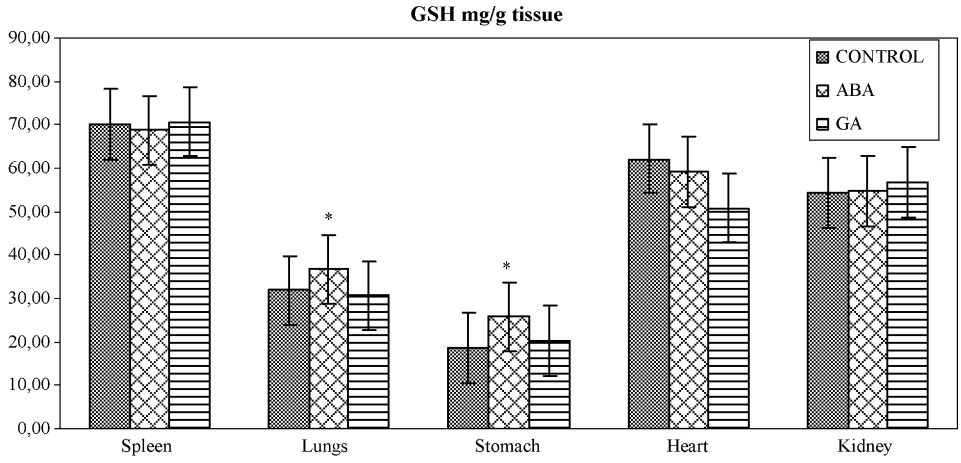

Fig. 1. Effects of subchronic treatment of ABA and GA on GR activity (U/g tissue) in the different tissues of rats. Values are means and ±S.D. * Significantly different from control rats at $P \leq 0.05$ (Mann–Whitney *U*-test).

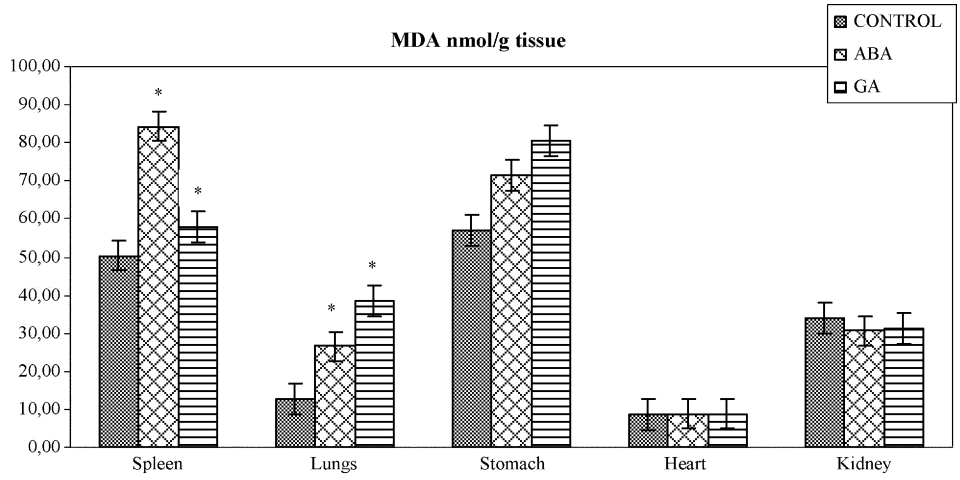

Fig. 2. Effects of subchronic treatment of ABA and GA on SOD activity (U/g tissue) in the different tissues of rats. Values are means and ±S.D. * Significantly different from control rats at $P \leq 0.05$ (Mann–Whitney *U*-test).

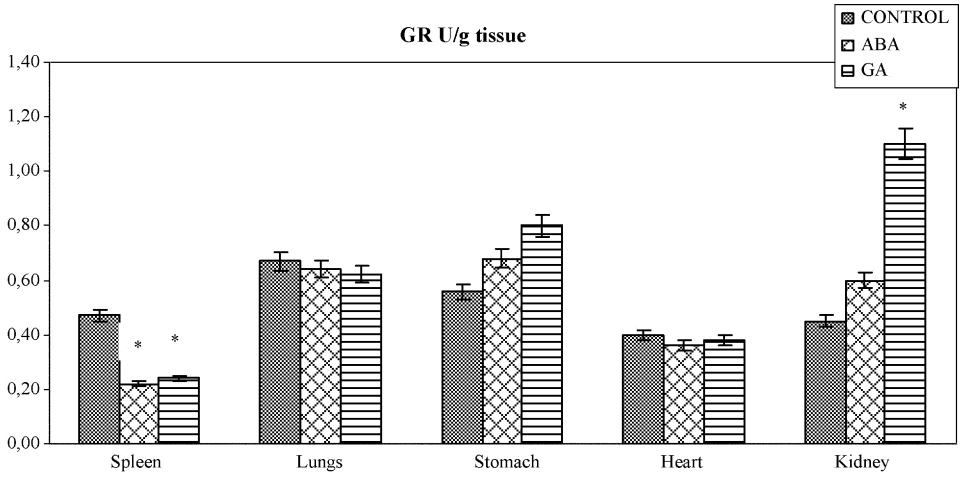

Fig. 3. Effects of subchronic treatment of ABA and GA on GST activity (U/g tissue) in the different tissues of rats. Values are means and ±S.D. * Significantly different from control rats at $P \leq 0.05$ (Mann–Whitney *U*-test).

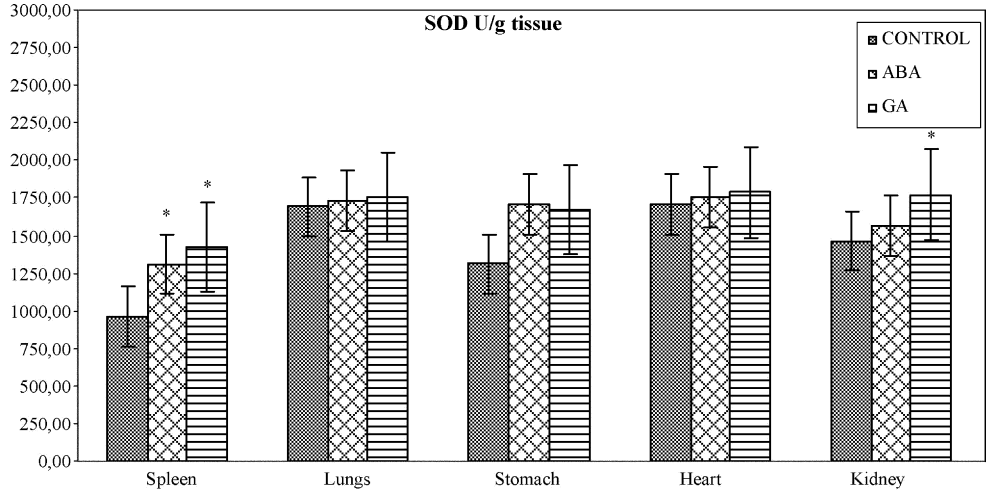

Fig. 4. Effects of subchronic treatment of ABA and GA on CAT activity (U/g tissue) in the different tissues of rats. Values are means and ±S.D. * Significantly different from control rats at $P \leq 0.05$ (Mann–Whitney *U*-test).

kidney. Also, ABA and GA_3 caused a significant increase in SOD activity in spleen. However, SOD activity increased in the kidney by GA_3. Furthermore, the GST activity was promoted by ABA and GA_3 in the spleen and lungs, but ABA caused a decrease in GST activity in heart. The changes in the other tissues were found not to be statically significant. On the other hand, antioxidant enzyme activities such as CAT significantly decreased in the lungs treated with ABA. CAT also decreased by the both PGRs in all rat tissues except for spleen. The decreases were not statistically significant. The reasons for such affect of PGRs are not understood at the present. But, PGRs may lead to the inhibition of the enzyme synthesis into tissues as a result of mRNA breakdown or cellular transcription mechanisms. Also, oxidative stress can affect the activities of protective enzymatic antioxidants in organisms exposed to the PGRs. 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 [32]. The increased activities of SOD, CAT, GPx, GR, and GST are known to serve as protective responses to eliminate reactive free radicals [33]. However, it is not a general rule that increases in pollutant concentrations induce antioxidant activity. Doyotte et al. [34] 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. Also, Dimitrova et al. [35] suggested that the superoxide radicals by themselves or after their transformation to H_2O_2 cause an oxidation of the cysteine in the enzymes and decrease in enzymatic activities. Further, this decrease in CAT activity could be due to the flux of superoxide radicals, which have been reported to inhibit CAT activity [36]. Consequently, the decreased CAT activity might have reflected a cellular oxidative stress due to ABA and GA_3 exposure, which have promoted the flux of superoxide radicals. Another possibility is that the PGRs may lead to the inhibition or stimulation of the enzymes synthesis in tissues by effect of mRNA transcription mechanisms. Nevertheless, the physiological roles of these antioxidant enzymes in the cell are poorly understood

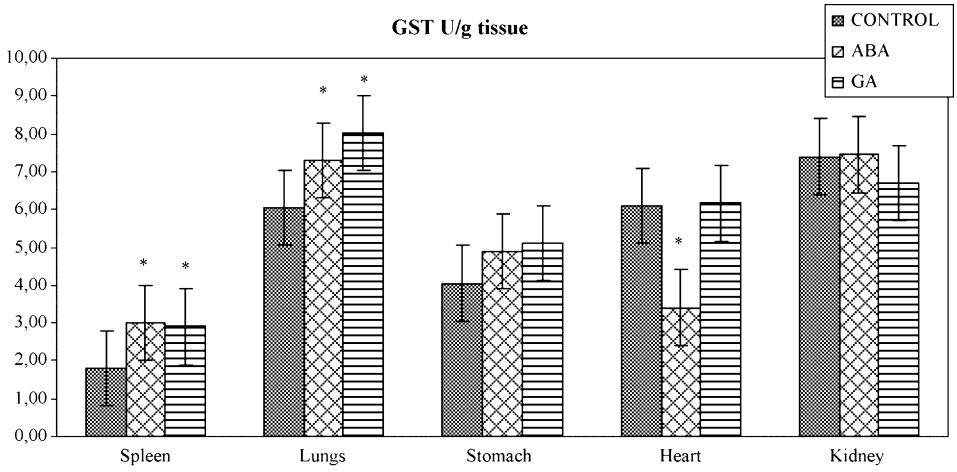

Fig. 5. Effects of subchronic treatment of ABA and GA on GSH levels (mg/g tissue) in the different tissues of rats. Values are means and ±S.D. *Significantly different from control rats at $P \leq 0.05$ (Mann–Whitney *U*-test).

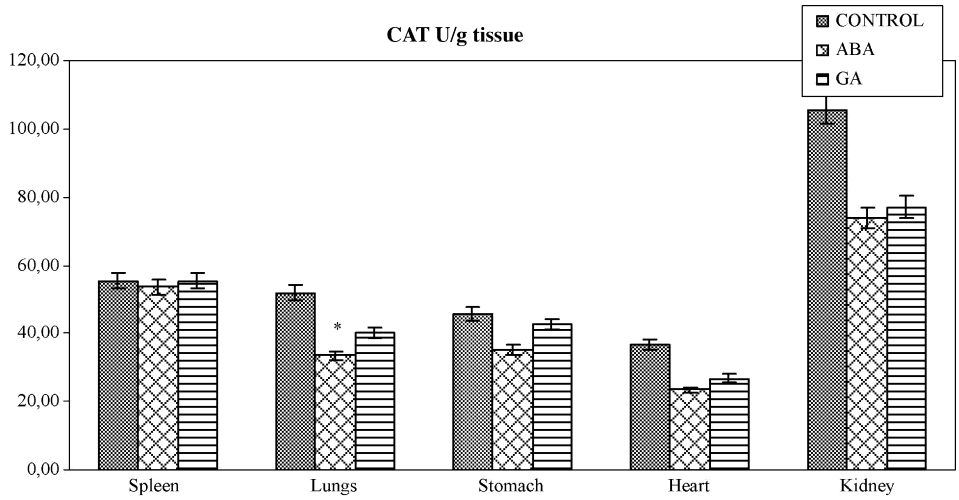

Fig. 6. Effects of subchronic treatment of ABA and GA on MDA content (nmol/g tissue) in the different tissues of rats. Values are means and ±S.D. * Significantly different from control rats at $P \leq 0.05$ (Mann–Whitney U-test).

because of complex interactions and interrelationships among individual components. The changes in the antioxidative systems presented in this study might indicate that the exposure of rats to ABA and GA_3 could have affected non-enzymatic antioxidant defense in vertebrates too. Results also showed that the GSH levels (Fig. 5) were significantly depleted in the lungs and stomach of rats treated with ABA without any change in all of the rest of tissues of rats treated with the both PGRs. GSH depletion might enhance the risk of the oxidative stress [36]. 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 reactive oxygen species generated from the PGRs. It is conceivable that ABA and GA_3 might be interacting primarily with the tissues, resulting in fluctuated enzymes activities by the way of increased free radicals as a result of stress condition in the rats. Reduced GSH and its metabolizing enzymes provide the major defense against ROS-induced cellular damage [37].

In addition to the fluctuated antioxidant defense systems, oxidative stress induced by the PGRs could have also affected the MDA content in organisms exposed to PGRs (Fig. 6). The lipid peroxidation end product MDA significantly increased in the spleen and lungs of rats treated with the both PGRs. The increased content of MDA might have resulted from an increase hydroxyl radical ($^{\bullet}OH$) as a result of stressed condition in the rats with the PGRs intoxication. It is known that $^{\bullet}OH$ can initiate lipid peroxidation in tissues [38]. However, it is conceivable that ABA and GA_3 might be interacting primarily with the tissues, resulting in lipid peroxidation processes by way of increase free 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]. 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 and MDA concentration in rat tissues. In addition, findings of this study suggest that the fluctuated levels in the constituents of antioxidant defense systems and MDA content in the tissues of rats exposed to PGRs may be dependent on the differences between interstitial concentrations. Namely, the systems might have to be exposed to different xenobiotic concentration due to blood volume differences in the tissues.

The observations presented led us to conclude that administration of subacute ABA and GA_3 promote MDA content and alter in the activities of antioxidative systems in the rat's various tissues. The test results may offer with means for monitoring toxicity of compounds such as PGRs and 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 PGRs toxicity.

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