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The toxic effects of a commercial herbicide in various tissues of wistar rats

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"Désormone Lourd" is a 2,4-Dichlorophenoxyacetic based herbicide that includes 600 g/L 2,4-D. In this study we analyzed the oxidative effects of 2,4-D on rat liver, kidney, plasma and erythrocytes. Animals were daily treated with 15, 75 and 150 mg/kg, via oral gavage during 4 weeks. Oxidative stress markers, catalase, superoxide dismutase and glutathione peroxidase (CAT, SOD and GPx) were analyzed. We also investigated lipid peroxidation by measuring thiobarbituric acid reactive substances (TBARs) and it was expressed in terms of malondialdehyde (MDA) content. Our results revealed that, when rats of 2,4-D treated groups were compared with the control group, the malondialdehyde level was significantly increased. Also, through sub-acute treatment, starting from the low to the high doses of 2,4-D, it was observed that there were significant effects on the activity of antioxidant enzymes (SOD, CAT and GPx). Pearson correlation analysis showed that oxidative effects were found in all tissues of the treated groups and their severity was dose dependent. To conclude, we can suggest that sub-acute exposure to 2,4-D induced oxidative damage in rats. Therefore, at higher doses, 2,4-D may be implicated in the pathogenesis of vascular diseases and other health related problems via lipid peroxidation and oxidative stress.

Keywords: 2,4-dichlorophenoxyacetic acid; rat; tissues; lipid peroxidation; oxidative stress.

Abbreviations

2,4-D, 2,4-Dichlorophenoxyacetic herbicide; **CAT**, Catalase; **GPx,** Glutathione Peroxidase; **LPO**, Lipid peroxidation; **MDA**, malondialdehyde; **ROS**, Reactive Oxygen Species; **SOD**, Superoxide Dismutase; **TBARs,** Thiobarbituric Acid Reactive substances.

INTRODUCTION

Pesticide exposure can lead to oxidative stress through unregulated generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical, peroxyl radicals and singlet oxygen. ROS are produced during normal process in the cell. Under normal conditions antioxidant systems of the cell minimize damage caused by ROS. When ROS generation increases to an extent that it overcomes the cellular antioxidant systems, the result is oxidative stress.

It is known that pesticides can cause oxidative stress, resulting in the generation of free radicals (Banerjee et al., 1999). It is suspected that pesticides induce alterations in antioxidants or free oxygen radical scavenging enzyme systems. In addition, it is generally believed that lipid peroxidation is one of the molecular mechanisms involved in pesticide induced toxicity (Akhgari et al., 2003). Indeed, Phenoxyherbicides stimulate generation/production of ROS. Selassie et al., (1998) suggests that this is related to two properties, one being the formation of free radicals from them, and the second being a direct attack of these phenoxyl radicals on biochemical processes in a number of sensitive metabolic pathways.

Among the Phenoxyacetic herbicides, 2,4-Dichlorophenoxyacetic acid (2,4-D), whose structural formula is shown in Figure 1, has been the most used since 1946. The toxicity of 2,4-D and other related compounds was attributed to the free acid form of the chemicals (Munro et al., 1992). It is known that it disturbs
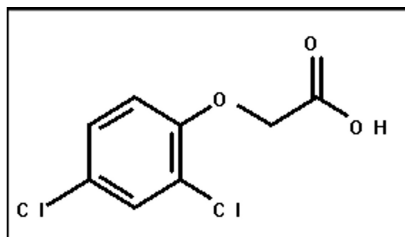
Figure 1. Chemical structure of 2,4-D

metabolism (Palmeira et al., 1995). Moreover, immunosuppressive (Pistl et al., 2003), neurotoxic (Bortolozzi et al., 2004) and hepatotoxic effects have been well documented (Tuschl and Schawb, 2003; Tayeb et al., 2010). As a phenoxyherbicide, 2,4-D may cause an array of adverse effects to the nervous system such as myotonia, disruption of the activity of nervous system and behavioural changes (Bortolozzi et al., 2004). In addition, it is known that 2,4-D provokes changes in the animal nervous system due to interaction with acetylcholinesterase (AchE) activity (Sarikaya and Yılmaz, 2003; Caglan et al., 2007; Cattaneo et al., 2008).

Herbicide 2,4-D has been suggested as a potential environmental endocrine disruptor and oxidative damage inducer (Munro et al., 1992; Mi et al., 2007). Several studies have shown that 2,4-D produces oxidative stress and/or depletes antioxidants both in vitro and in vivo. In vitro reports have looked, especially, at the effects of 2,4-D on hepatocytes and red blood cells (Palmeira et al., 1995; Bukowska, 2003). In vivo oxidative activity has been studied in many species including yeast, plants, fish and rats (Romero-Puerats et al., 2004; Teixeira et al., 2004; Oruc and Uner, 1999; Celik et al., 2006). Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity; as a consequence such pesticides can disturb the biochemical and physiological functions of some organs.

The aim of this study was to determine the effects on lipid peroxidation and antioxidant defence systems in liver, kidney, plasma and erythrocytes of wistar rats exposed to sub acute doses of Désormone lourd (commercial formulation of 2,4-D; 600 g L^{-1}).

MATERIALS AND METHODS

Chemicals

2,4-D commercial formulation (Désormone Lourd) consisting of 600 g/L 2,4-D Ester butylglycol, homologation number H.96064 (SEPCM), available in Tunisia, was used in experimentation. 2-Thiobarbituric acid (TBA) was obtained from Sigma chemical Co.(St. Taufkirchen, Germany). 1,1,3,3-tetramethoxypropane was purchased from Sigma chemical Co. (St. Louis, MO).

All other chemicals used were of analytical grade and were obtained from sigma chemicals Co or Merck (Darmstadt, Germany).

Animals

The current study was carried out in 40 male Wistar albino rats; weighing 150–180 g. the animals were obtained from Central Pharmacy (Société des Industries Pharmaceutiques Tunis, Tunisia). Before experiments, animals were housed for 2 weeks in polyethylene home cages, with sawdust-covered floors. They were maintained in a colony room at 22± 2° C under conditions of controlled humidity (70%±5%) and a 12 h light/dark cycle, with free access to water and standard commercial pellet chow (SICO, Sfax Tunisia). All breeding phases and all experiments were conformable to the rules of the Tunisian Society for the Care and Use of Laboratory Animals. All experiments were conducted at the animal facilities of the Faculty of Medicine, Monastir; with the approval of the Faculty of Medicine Ethics committee.

Experimental design

After the acclimatizing period, animals were randomly segregated into four different groups of 10 animals each. The control group received 1 ml of distilled water via oral gavage once a day. The 2,4-D treated groups – (G1), (G2) and (G3) – were respectively given 2,4-D at a dose level of 15, 75 and 150 mg/kg body weight (BW) in water, every day. Doses of 2,4-D for each animal was placed into a syringe that was inserted orally with the help of gastric drill directly into the oesopharyngeal region. All the treatments continued for a period of 4 weeks. 2,4-D solutions were administered in the morning (between 8:00 and 9:00) to non-fasted rats. Before sacrificing the animals, they were fasted overnight.

Blood and organ tissue collection

Blood samples were withdrawn from the animals after 28 days of treatment under ether anesthesia by cardiac

puncture using syringe. Some blood samples were collected into evacuated tubes containing EDTA solution as anticoagulant for determination of hemoglobin (Hgb) value. The other ones were collected into heparinised tubes for dosage of antioxidant enzymes activities.

Liver and kidney samples were taken by surgical processing after animal was killed. organs were rinsed in ice cold isotonic saline (0.9% w/v NaCl), blotted dry, and weighed separately. A 10% (w/v) tissue homogenate was prepared in ice-cold 10 mmol/L phosphate-buffered saline (pH 7.4) containing 1.15 % KCl. The homogenate was subjected to centrifugation at 6500 rpm for 15 minutes at 4 °C. The supernatant fractions were collected and stored at -80 °C until analysis.

Erythrocyte preparation and plasma collection

Within 30 min of blood collection, the erythrocytes were sedimented by centrifugation (600g, 10 min at 4 °C) then the plasma was removed and stored at -80 °C in aliquots until analysis. The sediments containing erythrocytes were suspended in phosphate buffer saline (0.9% NaCl in 0.01 M phosphate buffer, pH 7.4) and centrifuged as reported by Sinha et al. (2007). This process was repeated twofold. After removing cell's debris by centrifugation (3000g for 15 min) the hemolysats were obtained and stored at -80 °C until measurements.

Activities of antioxidant enzymes

Analyses of antioxidant enzyme activities were made by using a BioRad UV-Visible spectrophotometer with a "kinetics" program (BioRad, Mares la Coquette, France). The measurement of Superoxide dismutase (SOD) activity was performed by using commercially available diagnostic kits supplied by Randox Laboratories based on the method of Wooliams et al. (1983). The principle of the technique is as follows: xanthine reacts with xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction.

The determination of Glutathione Peroxidase (GSH-Px) activity was performed using Ransel reagents (Randox Laboratories) and is based on the method of Paglia and Valentine (1967). Glutathione peroxidase catalyzes the oxidation of reduced glutathione by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance of NADPH was measured at 340 nm.

The catalase activity was measured at 20 °C according to a slightly modified version of Aebi's method (1984). The rate of H_2O_2 decomposition was followed by monitoring absorption at 240 nm. One unit of CAT activity is defined as the amounts of enzymes required to decompose 1 μmol of hydrogen peroxide in 1 min. the molar extinction coefficient of 43.6 $mol.L^{-1}.cm^{-1}$ was used to determine CAT activity.

TBA-reactive substances assay

The level of lipid peroxidation products was measured as TBA-reactive metabolites according to the method of Yagi (1976). One hundred twenty-five microliters of serum or supernatant obtained from organs was homogenized by sonication with 50 mL of Tris buffered saline, 125 ml of TCA-BHT to precipitate proteins, and then centrifuged (1000 x g, 10 min, 4 °C). Two hundred microliters of the supernatant was mixed with 40 mL of HCl (0.6 M) and 160 mL of TBA dissolved in Tris and the mixture was heated at 80 °C for 10 min. The absorbance of the resultant supernatant was read at 530 nm. The TBA-reactive metabolites amount was calculated using a 156 mmol/cm extinction coefficient.

Protein assay

Protein content in plasma and supernatant fractions was determined according to Bradford method (1976). Absorbance was measured spectrophotometrically at 750 nm.

Determination of erythrocyte hemoglobin levels

Erythrocyte hemoglobin levels were determined as described by Drabkin (1946). The indicated method is based on the generation of cyanomethemoglobin during analysis and the measurement of it at 540 nm.

Statistical analyses

In each assay, the experimental data represent the means of ten independent assays (n = 10) ± standards deviations. Data were analyzed using SPSS 11.0 for Windows (Chicago, IL). The statistical significance has been determined using one-way analysis of variance (ANOVA). Correlation coefficients were calculated using the procedure of Pearson. Differences were considered significant at the level $p < 0.05$ and very significant at the level $p < 0.01$. Means comparison was done using Duncan Test.
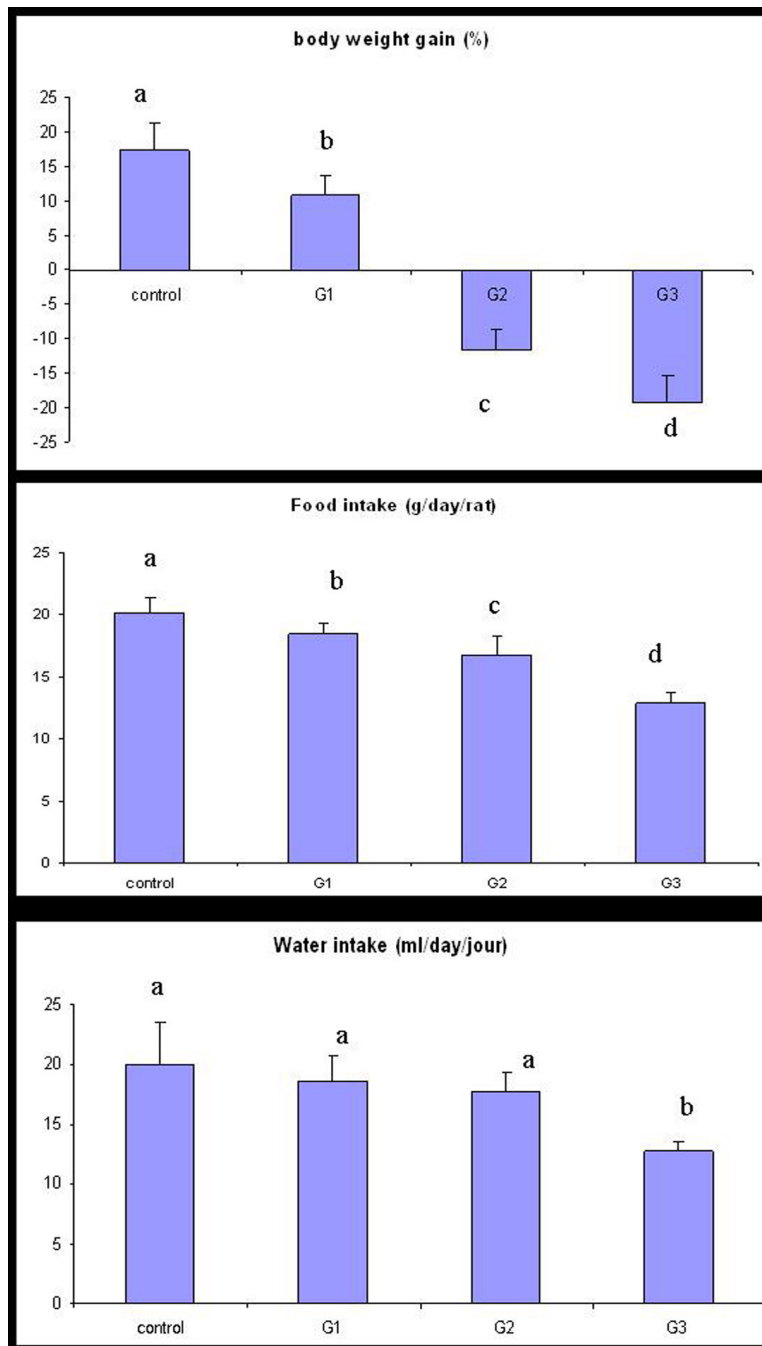
Figure 2. Body weight gain, food and water consumption of control and experimental rats

RESULTS

Effects of 2, 4-D treatment Body weight gain, daily food and water consumptions

2,4-D herbicide, at the three studied doses, caused significant alterations in Body weight gain, daily food and water consumptions in comparison to those of controls. In fact, the results in Figure 2 show that 2,4-D treatment induced a decrease in body weight gain (10.90, -11.78 and -19.40 %), drinking water (6.66, 11.54 and 36.13%) and food (8.67, 17.10 and 36.04%) consumptions, respectively for 15, 75 and 150 mg/kg of 2,4-D treated rats.

Table 1. Effect of a subacute exposure with 2,4-D, given for 4 weeks on the activity of superoxide dismutase in rat tissues.

Parameters	Control	2,4-D treatment		
		G1	G2	G3
Plasma (U/g protein)	12.92 ±1.98[b]	8.95 ±1.42[a]	8.14 ±1.27[a]	7.38 ±0.56[a]
Liver (U/mg protein)	8.54 ±0.24[b]	11.73 ± 2.1[c]	6.18 ± 0.48[a]	5.23 ±1.07[a]
Kidney (U/mg protein)	5.29 ±0.55[a]	3.3 ± 0.33[b]	4.54 ± 0.49[b]	2.76 ± 0.7[c]
Erythrocytes (U/g Hgb)	767.29 ±177.56[a]	564.19 ± 71.34[b]	391.31 ± 69.86[c]	323.76 ± 64.13[c]

Data are expressed as means ± S.D (n= 10 rats per group). Values on the same line with different letters are significantly different at *p<0.05*.
"control" indicates control rats treated daily with 1 ml of distilled water; "G1", rats treated with 15 mg/kg BW of 2,4-D; "G2", rats treated with 75 mg/kg BW of 2,4-D; " G3", rats treated with 150 mg/kg BW of 2,4-D.

Table 2. Effect of a subacute exposure with 2,4-D, given for 4 weeks on the activity of glutathione peroxidase in rat tissues.

Parameters	Control	2,4-D treatment		
		G1	G2	G3
Plasma (U/g protein)	176.63 ± 38.68 [b]	130.93 ± 46.92 [ab]	122.47 ± 30.92 [ab]	112.87 ± 16.14 [a]
Liver (U/mg protein)	0.85 ± 0.1[c]	0.36 ± 0.08[b]	0.12 ± 0.01[a]	0.31 ± 0.09[b]
Kidney (U/mg protein)	0.86 ± 0.18[a]	0.65 ± 0.13[a]	0.64 ± 0.13[a]	0.37 ± 0.09[b]
Erythrocytes (U/g Hgb)	639.17 ± 62.23[a]	290.31 ± 61.16[b]	211.04 ± 48.32[c]	185.45 ± 37.74[c]

Data are expressed as means ± S.D (n= 10 rats per group). Values on the same line with different letters are significantly different at *p<0.05*.
"control" indicates control rats treated daily with 1 ml of distilled water; "G1", rats treated with 15 mg/kg BW of 2,4-D; "G2", rats treated with 75 mg/kg BW of 2,4-D; " G3", rats treated with 150 mg/kg BW of 2,4-D.

Table 3. Effect of a subacute exposure with 2,4-D, given for 4 weeks on the activity of catalase in rat tissues.

Parameters	Control	2,4-D treatment		
		G1	G2	G3
Plasma (mmol/min g protein)	711.45 ± 175.15 [b]	487.2 ± 140.89 [a]	508 ± 105.8 [a]	447.5 ± 70.67 [a]
Liver (μmol/min/ mg protein)	23.42 ± 0.54[c]	19.47 ± 2.27[b]	17.87 ± 0.93[b]	13.3 ± 2.23[a]
Kidney (μmol/min/ mg protein)	19.92 ± 1.82[a]	14.33 ± 3.79[b]	16.79 ± 1.99[b]	11.85 ± 1.04[c]
Erythrocytes (U/g Hgb)	1565.53 ± 397.4[a]	1052 ± 182.13[b]	1013.60 ± 120.8[b]	989.27 ± 65.08[b]

Data are expressed as means ± S.D (n= 10 rats per group). Values on the same line with different letters are significantly different at *p<0.05*.
"control" indicates control rats treated daily with 1 ml of distilled water; "G1", rats treated with 15 mg/kg BW of 2,4-D; "G2", rats treated with 75 mg/kg BW of 2,4-D; " G3", rats treated with 150 mg/kg BW of 2,4-D.

Effects of 2, 4-D treatment on activities of antioxidant enzymes and lipid peroxydation

The results of experiment showed that the treatment of rats with 2,4-D, changed MDA contents, and antioxidant enzymes such as CAT, GPx and SOD in the erythrocyte, liver, kidney and plasma in comparison to control rats (Table 1-4).

Table 5 illustrates the Pearson correlations between biological markers (SOD, CAT, GPx and TBARs) and the

Table 4. Effect of a subacute exposure with 2,4-D, given for 4 weeks on the level of TBARs in rat tissues.

Parameters	Control	2,4-D treatment		
		G1	G2	G3
Plasma (µmol/g protein)	0.02 ± 0.009^a	0.05 ± 0.009^b	0.06 ± 0.014^c	0.08 ± 0.006^c
Liver (µmol/g protein)	0.58 ± 0.02^a	1.06 ± 0.24^b	1.51 ± 0.11^c	1.75 ± 0.16^c
Kidney (µmol/g protein)	0.7 ± 0.14^a	1.36 ± 0.34^{ab}	1.93 ± 0.72^b	2.07 ± 0.53^b

Data are expressed as means ± S.D (n= 10 rats per group). Values on the same line with different letters are significantly different at *p<0.05*.
"control" indicates control rats treated daily with 1 ml of distilled water; "G1", rats treated with 15 mg/kg BW of 2,4-D; "G2", rats treated with 75 mg/kg BW of 2,4-D; " G3", rats treated with 150 mg/kg BW of 2,4-D.

Table 5. Overall correlations between dose of 2,4-D administered – and biological markers in rat tissues.

	2,4-D Dose	SOD	CAT	GPx	TBARs
2,4-D Dose	-				
SOD	-0.624*	-			
	-0.489				
	-0.790**				
	-0.861**				
CAT	-0.929**	0.466	-		
	-0.669**	0.757**			
	-0.514*	0.754**			
	-0.627**	0.569*			
GPx	-0.756**	0.276	0.633*	-	
	-0.784**	0.584*	0.685**		
	-0.637*	0.770**	0.639*		
	-0.862**	0.776**	0.754**		
TBARs	0.950**	-0.547	-0.850**	-0.812**	-
	0.793**	-0.464	-0.702**	-0.555*	
	0.882**	-0.813**	-0.514*	-0.565*	

In each table cell, the first value correspond to Pearson coefficient calculated for liver samples, the second value for kidney, the third value for plasma and the forth for erythrocytes samples. * p<0.05 ; ** p<0.01

dose of 2,4-D administered to rats. It was interesting to note that over the dose of 2,4-D is higher, significant correlations between all biomarkers were found in all of the rat's tissues. In fact, we found that the dose of 2,4-D correlated positively with TBARs, in turn increase in the dose of this herbicide showed an inverse correlation with modifications in SOD, CAT and GPx in all of the rat's tissues. Also, the Pearson correlation analysis (Table 5) showed significant correlations between all tissues biological markers.

DISCUSSION

In recent years, a significant increase in the use of pesticides against harmful agricultural pests has been observed in Tunisia as well as in the rest of the world. In the present study, 2,4-D, was preferred because information on its negative effects on higher animals is limited for in vivo oral exposures. Also, 2,4-D is 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 2,4-D, increased the production of lipid peroxides, and affected antioxidant defence of various rat tissues.

So far, regarding the toxicological effect of 2,4-D, it will be difficult to compare the present results with the previous data 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. For the extent of chemical effect too, little is known about the biochemical or physiological effects in the vertebrates.

In the present study, we have noted that rats exposed to 2,4-D had lower body weight than those of controls (Table 1). This could be due to a reduction of food and water intake by treated rats. In a study done by Lee et al. (2001), authors confirm that 2,4-D herbicide may alter a variety of host physiologic and pathologic conditions such as nutritional status and so affect the gain of mice body weight.

The results of the current study have demonstrated that a subacute treatment with three increasing doses of

2,4-D could have affected MDA content and antioxidant defence systems in rats. This is evidenced from our observation that, upon 2,4-D treatment in vivo, the concentration of MDA, and the antioxidative markers in the liver, kidney, plasma and erythrocytes differ from that of controls. The pearson correlation analysis showed that the dose of 2,4-D correlated positively with TBARs in all of the rat's tissues. The increased content of MDA by the studied herbicide may result from reactive oxygen species (ROS). It is well known that the elevation of lipid peroxidation after some xenobiotic is consumed, and followed often by the superoxide overproduction, which after dismutation produce singlet oxygen and hydrogen peroxide, and it 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. Also, it is known that OH can initiate lipid peroxidation in tissues (Halliwell and Gutteridge, 1989) and MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation (Freeman and Crapo, 1981).

Antioxidant enzymes namely catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) are the first line of defence against oxidative stress. These enzymatic antioxidants 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 (Rodriguez-Ariza et al., 1993). The effects of 2,4-D is likewise too. Especially, that according to our investigation, it was interesting to note that as the dose of 2,4-D administred is important, significant correlations between several biological markers were found. So, such biomarkers might be used to detect the early biochemical effects of 2,4-D before adverse clinical health effects occur.

In fact, Oxidative stress can affect the activities of protective enzymatic antioxidants in organisms exposed to phenoxyherbicides such as the 2,4-D (Tayeb et al., 2012). Indeed, in our study we have noted that subacute treatment with three increasing doses of 2,4-D could have affected the antioxidant defence systems in rats ; also the Pearson correlation analysis (Table 5) showed significant inverse correlations between all tissues antioxidant enzymes and the dose of 2,4-D administred. According to Amstad et al., (1991), the decreased activity of antioxidative enzymes may lead to decrease the protection against oxidants especially that decrease in the activities of these enzymes changes the redox status of the cells. Doyotte et al. (1997) pointed out that a decreased response may accompany a first exposure to pollutants, which can be followed by an induction of antioxidant systems. Also, Dimitrova et al. (1994) suggested that the superoxide radicals by themselves or

after their transformation to H2O2 cause an oxidation of the cysteine in the enzymes and a decrease in enzymatic activities. The decreased SOD activity may reflect a cellular oxidative stress due to PGRs exposure. According to Halliwell and Gutteridge (1989), 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. Further, the decrease in CAT activity could be due to the flux of superoxide radicals, which have been reported to inhibit CAT activity (Regoli and Principato, 1995). So, the decreased CAT activity might have reflected a cellular oxidative stress due to subacute 2,4-D exposure, which has promoted the flux of superoxide radicals. Another possibility presented by Celik and Tuluce (2007), is that plant growth regulators as 2,4-D may lead to the inhibition of the enzymes synthesis in tissues by effect of mRNA transcription mechanisms. Nevertheless, the physiological roles of the antioxidant enzymes in the cell are poorly understood because of complex interactions and interrelationships among individual components.

However, our results are partly in accordance with previous results reported by Celik et al. (2006), Celik and Tuluce (2007); Troudi et al. (2011) despite the differences between studies in their settings, materials and experimental designs. All of these studies confirm that 2,4-D seems to induce in vivo oxidation only with high doses and with increasing length of administration period. It is known that phenoxyacetic acid herbicides are eliminated by a renal anion transport system which is saturated as plasma concentration increases. Since saturation of the rodent renal transporter is reported to occur at doses in excess of 50 mg/kg/day, then the rise in blood concentration as dose of herbicide increases may lead to the distribution of the compound into cells and tissues which then become susceptible to oxidative stress. So, this may account for our current observations of oxidation being induced in rat's plasma, red blood cells, liver and kidney tissues (Figure 3).

In the literature, the role of oxidative stress has been widely implicated in the incidence of cardiovascular disorders, hypertension and other health related problems (Singh et al., 2007). Indeed, pesticides as 2,4-D have been implicated in the pathogenesis of such disorders (Kang et al., 2006; England, 1981; Schreinemachers, 2010). Thus, we have been concerned about the possible implication of 2,4-D in the pathogenesis of vascular failure. Hence a series of studies were undertaken to assess the protective effect of repeated consumption of olive oil on 2,4-D induced tissue damage in rats. Our recent finding suggests that including olive oil in the diet may offer benefits in decreasing tissue damage and the atherosclerotic process during 2,4-D exposure in rats (Nakbi et al. (2010a; 2010b; 2011; 2012).
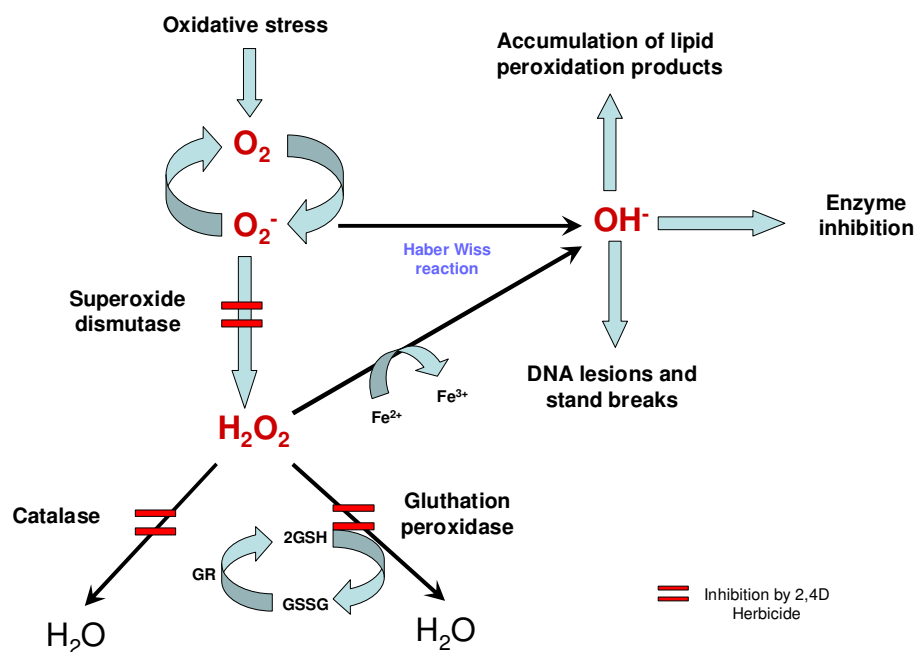
Figure 3. Mechanisms underlying the toxicity of 2,4-D

CONCLUSION

As a conclusion, the results indicate that administrations of subacute 2,4-D might affect on antioxidative defence systems and promote MDA content in the rat's plasma, erythrocytes, liver and kidney tissues. Also, significant correlations between SOD, CAT, GPx and TBARs were found. So, such biomarkers might be used to detect the early biochemical effects of 2,4-D before adverse clinical health effects occur.

In addition, findings of the current study suggest that further experiments should be performed to elicit what is responsible for the decreasing activities of antioxidant and induce MDA concentration in rat tissues.

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