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Oxidative Stress Induced by the 2,4-Dichlorophenoxyacetic Herbicide

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1. Introduction

Phenoxyacetic herbicides constitute one of the largest groups of herbicides sold in the world. Among them, since 1946, 2,4-Dichlorophenoxyacetic acid (2,4-D), whose structural formula is shown in Fig. 1, has been the most used. Nowadays, new formulations of 2,4-D are continuously made available. In fact, there are over 600 2,4-D products currently on the market. For over 60 years, 2,4-D has been the most commonly and widely used herbicide throughout the world (Tayeb et al., 2011 a).

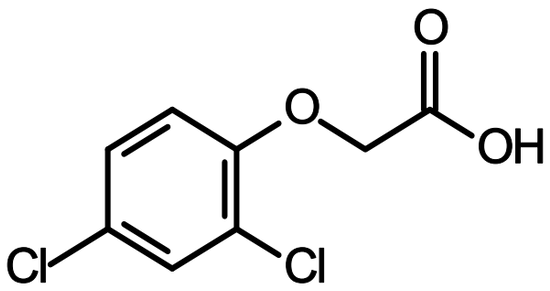

Fig. 1. Chemical structure of 2,4-D

2,4-D is primary used as a weed control in agriculture, forestry, and lawn care practices. It is marked as a selective systemic herbicide. Its herbicidal activity is mediated by an auxin-like capacity to alter normal protein synthesis and cell division in plant meristems and leaves (Stevens and Breckenridge, 2001). While at low concentrations 2,4-D acts as an auxin analogue promoting plant growth, at high concentrations it is lethal and used as herbicide against broad-leafed and woody plants (Mullison, 1987). Yet, Romero-Puertas et al. (2004) have recently suggested that the 2,4-D- herbicidal activity may also be due to an increase in the production of oxygen reactive species (ROS). The latter's lead to the generation of oxidative stress in the weed.

Upon application, 2,4-D is distributed into various compartments of the environment in accordance with its physical/chemical properties and local environmental conditions (Tayeb et al., 2011a). 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 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 behavioral 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., 2008; Cattaneo et al., 2008).

A review of the toxicology and mechanism of toxicity of 2,4-D is necessary to assess the potential risk for animal and human health. Literature on the induction of oxidative stress and involvement of lipid peroxidation after 2,4-D in vitro and in vivo exposure will be reviewed here to provide an updated scientific basis to derive future research studies on this compound. Included in the review was information on the possible implication of 2,4-D exposure in the pathogenesis of health problems; and, a survey of current studies dealing with the antioxidant properties of some substances to decrease the oxidative stress induced by 2,4-D.

2. The 2,4-D herbicide and oxidative stress

Although the exact mechanisms by which this herbicide is incorporated into cells are not totally understood, 2,4-D has been reported to be a peroxisome proliferator (Bradberry et al., 2000). In plant cells 2,4-D induces mitotic and meiotic irregularities both in vivo and in vitro (Khalatkar and Bhargava, 1982). In mammalian cells in vitro, 2,4-D inhibits cell growth, protein and DNA synthesis, and arrests cells in the G/S phase of the cell cycle (Rivarola et al., 1985). Later, Maire et al. (2007), in their study on mammalian cells, showed that DNA damage detected by the comet assay could be related to oxidative stress. 2,4-D was found to induce oxidative stress, a mechanism responsible for DNA damage measured by the comet assay in fish (Martinez-Tabche et al., 2004). The induction of oxidative stress leading to secondary genotoxicity was proposed as a possible mechanism for carcinogenicity (Beddowes et al., 2003).

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.

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.

2.1 *In vitro* studies

To study the in vitro effects of pesticide exposure, several researchers use biological lipid membranes model like erythrocyte ghosts as they are sensitive to the peroxidative process; since they are rich in polyunsaturated fatty acids in their membranes, a class of compounds highly susceptible to lipid peroxidation. The majority of works dealing with the effects of 2,4-D and its metabolites on erythrocytes were summarized in table 1. Toxic influence of 2,4-D may provoke disturbances in bilayer phospholipid structure that plays an important role in the correct function of cell membrane. Phenoxyherbicides interact with proteins and lipids of erythrocyte membrane (Suwalsky and Berites, 1996). Indeed, Janik and Wolf (1992) have demonstrated the inhibitory effect of chlorinated compounds on the Ca-ATPase which indicates a toxic effect to human erythrocytes functions. Bukowska et al. (1998) have found the increase in the level of methemoglobin (metHgb) and the change of the oxygen affinity of haemoglobin under the influence of 2,4-D. Later, Bukowska (2003) reported that treatment of human erythrocytes in vitro with 2,4-D at 250 and 500ppm resulted in decreased levels of reduced glutathione, decreased activity of superoxide dismutase, and increased levels of glutathione peroxidase. These significant changes in antioxidant enzyme activities and evidence of oxidative stress indicate that 2,4-D should be taken seriously as a cytotoxic and potentially genotoxic agent. In 2008, Bukowska et al. present the evidence for a direct prooxidant activity of phenoxyherbicides. In fact, the pro-oxidative action of these compounds is strongly dependent on the localization of the substituent in the phenol ring. Indeed, the compounds with chlorine residues in the second and fourth position of phenol ring cause strong damage to antioxidative enzymes and lipid peroxidation (Bukowska, 2003; Bukowska et al., 2000; Duchnowicz et al., 2002). Also, they much more easily penetrate the cell membrane. Bukowska et al. (2008) proposed a metabolic reaction chain that explains the mechanism of action of 2,4-D in vivo. The authors have noted that the prooxidative capability of this herbicide are related with its hydrolysis to 2,4-dichlorophenol that may generate radicals oxidizing H_2DCF, marker of oxidative status of the cells.

Other in vitro studies, dealing with the induction of oxidative stress after 2,4-D exposure, were conducted on hepatocytes. Palmeira et al. (1994) suggested that 2,4-D can decrease ATP, GSH and NADH levels while conversely increasing the levels of AMP, NAD, LDH and GSSG in rat hepatocytes. This herbicide at (1- 10 mM) may induce cell death by decreasing cellular GSH/GSSG ratio, promoting loss of protein thiol contents and inducing lipid peroxidation (Palmeira et al., 1995). In fact, it is suggested that membrane protein thiols can be attacked by radicals, resulting in a membrane protein thiol loss which in turn may also be associated with the development of hepatocellular injury.

Parameter	2,4-D	References
Decreased activity of CAT	- Effects observed at 1000 ppm (24 hours)	Bukowska, 2000
Induction of hemolysis Lipid peroxidation	- Effects observed at 1mM	Duchnowicz et al., 2002
Oxidation of haemoglobin	- Effects observed at 0.5 mM	Duchnowicz et al., 2002
Increase in membrane fluidity	- Effects observed at 1 mM	Duchnowicz and Koter, 2003
Glutathione peroxidise activity	- Effects observed at 1.13 mM	Bukowska et al., 2003
Depletion of GSH level Increased activity of GSH-Px Decreased activity of SOD	- Effects observed at 500 ppm - Effects observed at 250 ppm - Effects observed at 250 ppm (1hour)	Bukowska, 2003
W/S parameter that reflect denaturation or protein conformational in membrane	- Effects observed at 2 mM	Duchnowicz et al., 2005
Carbonyl group content Oxidation of H_2DCF	- Effects observed at 1.13 mM - Effects observed at 1.13 mM (3 h incubation)	Bukowska et al., 2008

Table 1. Effects of 2,4-D on human erythrocytes

2.2 *In vivo* studies

Studies in order to understand the toxic mechanism of 2,4-D in living cells have been performed using, e.g., Saccharomyces cerevisiae (Teixeira and Sa-Correia, 2002). Indeed, yeast has proved to be a useful experimental model for the study of basic molecular mechanisms underlying the toxicological effects of the important agrochemical 2,4-D and the associated adaptive responses (Papaefthimiou et al., 2004).

2.2.1 Yeast

At low pH (e.g. acidic soils, the alimentary canal of animals), the highly lipophilic weak acid 2,4-D exists in its undissociated lipophilic toxic form (RCOOH), which can readily cross the plasma membrane by passive diffusion. In the neutral cytosol, the undissociated form of 2,4-D dissociates, leading to internal acidification (Simoes et al., 2003; Fernandes et al., 2003) and to accumulation of the toxic counter-ion ($RCOO^-$), which cannot easily cross the plasma membrane lipid bilayer (Figure 3). Therefore, at low pH the toxic potential of the herbicide increases dramatically (Cabral et al., 2003).

Herbicide accumulation in the yeast cell leads to a dose-dependent increase in the level of hydroxyl radicals, as detected using in vivo electron paramagnetic resonance (EPR) spectroscopy (Teixeira et al., 2004). A coordinate transient increase in hydroxyl radical and lipid peroxidation levels was registered as a consequence of acute 2,4-D stress (Figure 2).

Results from of the early yeast response to 2,4-D provided additional clues to its possible mode of pro-oxidant action (Table 2). The response to 2,4-D includes the upregulation of genes involved in peroxisomal beta oxidation and mitochondrial oxidative phosphorylation, two metabolic processes leading to the endogenous generation of reactive oxygen species (ROS). Electron leakage from the mitochondrial respiratory chain might further increase the level of ROS generated during short-term cell exposure to 2,4-D (Figure 2).

The global yeast response to 2,4-D, revealed by microarray and proteomic analyses, indicates the upregulation of a large number of genes involved in alternative carbon and nitrogen source metabolism (Teixeira et al., 2006a) and in the uptake and biosynthesis of amino acids (Teixeira et al., 2006a; Teixeira et al., 2005), correlating with a dramatic reduction of the intracellular concentration of amino acids (Teixeira et al., 2005). These adaptive mechanisms might be a response to the deleterious effects exerted by 2,4-D on plasma membrane lipid organization and permeability, leading to nutrient import inhibition (Bradberry et al., 2000) (Figure 2).

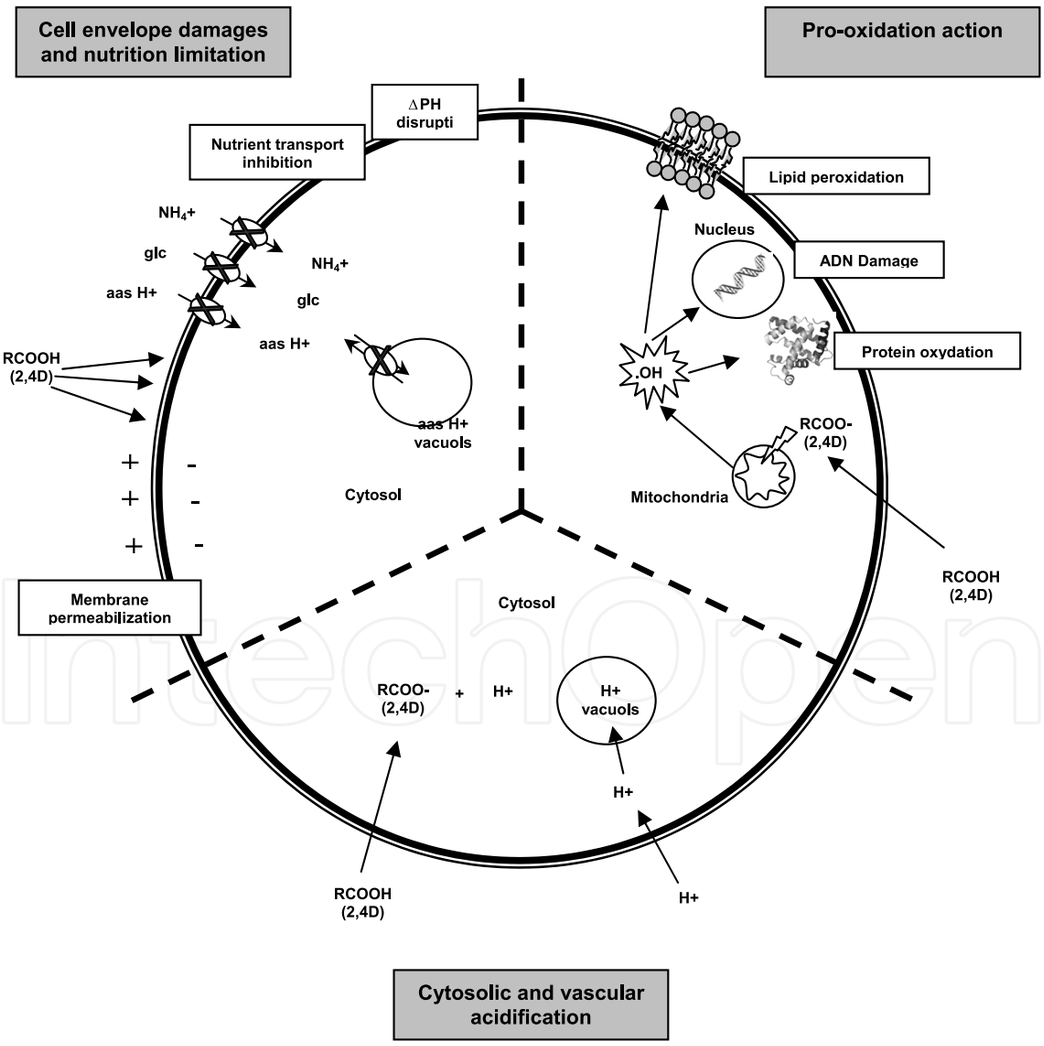

Fig. 2. Model for the mechanisms underlying the toxicity of 2,4-D in the yeast

Moreover studies on S. cerevisiae, Benndorf et al. (2006) studied the responses of Pseudomonas putida to chlorophenoxy herbicides. They described the induction of Stress proteins by antioxidant enzymes in the organisms P. putida KT2440 (cells) when stressed with 2,4-D. Also, 2,4-D induced oxidative stress in spermatogonial cells (Mi et al., 2007). In fact, exposure to 2,4-D elicited TBARS products of lipid peroxidation, and decreased glutathione content and SOD activity in embryonic chickens. In 2010, Park et al. studied the Biological and molecular responses of Chironomus riparius (Diptera, Chironomidae) to herbicide 2,4-D. Their results showed that the responses of HSPs and GST in C. riparius exposed to 2,4-D suggest that it can induce oxidative damage and changes in the endocrine system. The authors found that the upregulation of ferritin genes in C. riparius exposed to 2,4-D may lead to protection responses against 2,4-D induced oxidative damage.

Effects observed after 2,4-D exposure	**Reference**
- Production of many stress and heat-shock proteins during the adaptation period, and that eventually cell division occurred in the presence of 2,4-D.	Teixeira and Sa-Correia, 2002
- Coordinated stimulation of vacuolar and plasma membrane H^+-ATPase activities, to counteract the dissipation of the physiological H^+-gradients across vacuolar and plasma membranes occurring under 2,4-D stress.	Fernandes et al., 2003
- The transient increase in free radical (hydroxyl radicals) generation and lipid peroxidation in the yeast cell challenged with 2,4-D correlates with the stimulation of the activity of antioxidant enzymes (Ctt1p, Sod1p, Grx1p and Grx2p).	Teixeira et al., 2004
- Increased content of Vma1p and Vma2p (two submits of vacuolar H^+-ATPase).	Teixeira et al., 2005
- Changes at the level of membrane lipid composition in yeast cells adapted to 2,4-D, including an increase in the saturation degree of membrane fatty acids.	Viegas et al., 2005
- Upregulation of genes involved in peroxisomal β-oxidation and mitochondrial oxidative phosphorylation, two metabolic processes leading to the endogenous generation of ROS	Teixeira et al., 2006a
- Identification of Msn2p and Msn4p as the putative transcriptional regulators of 20% of the 2,4-D-activated genes. These target genes encode heat shock proteins, molecular chaperones and antioxidant enzymes	Teixeira et al., 2006b

Table 2. Effects of 2,4-D on yeast

2.2.2 Fish

On fish, at tissue level, the 2,4-D toxicity follow the common route through the gills and external tegumenta and by the digestive tract to a small extent. The absorbed chemical has

been shown to bind proteins of plasma so as to be transported throughout the organisms (Arnold and Beasley, 1989). Structural abnormalities like vacuolation of erythrocytes is a regular feature of 2,4-D (Ateeq et al., 2002) like many other chemicals.

Several studies showed that the antioxidants of fish may be useful biomarkers of exposure to aquatic pollutants as 2,4-D herbicide (Ahmad et al., 2000). Table 3 present the most important works done in this field. The study of Ozcan Oruc and Uner (2000) aims to investigate the effects of the herbicide 2,4-D and the insecticide azinphosmethyl on hepatic antioxidant enzyme activities and lipid peroxidation in tilapia. Fish were exposed to 27 ppm 2,4-D, 0.03 ppm azinphosmethyl and to a mixture of both for 24, 48, 72 and 96 h. It was concluded that the metabolism of pesticide-exposed *O. niloticus* resisted the oxidative stress using the antioxidant mechanism and prevented the increase of lipid peroxidation. Later on 2004, these authors studied the tissue-specific oxidative stress responses in fish exposed to 2,4-D and azinphosmethyl. Results indicate that the toxicities of azinphosmethyl and 2,4-D may be related to oxidative stress. In fact, this last study revealed that fish exposed to pesticides develop tissue-specific adaptive responses to protect cells against oxidative stress. Moreover, according to our results, the elevations in gill SOD activity and kidney GST activity serve as biomarkers of oxidative stress and may be helpful in assessing the risk of environmental contaminants. Also, Zhang et al. (2004) explored the hepatic antioxidant responses of fish *Carassius auratus* to long-term exposure of 2,4- dichlorophenol. They concluded that SOD and Se-GPx may be potential early biomarkers of 2,4-DCP contamination in aquatic ecosystems.

Specie	**Organ**	**Dose**	**Results**	**References**
O. niloticus	liver	27 ppm for 24, 48, 72 and 96 h	Increase in GPx (for 96h) and GR activities	Ozcan Oruc and Uner, 2000
O. niloticus	kidney	87 ppm for 96h	Increase in GPx and GST activities	Ozcan Oruc and Uner, 2004
	Gill		Increase in SOD activity	
	Brain		Decrease in GPx activity	
C.carpio	kidney	87 ppm for 96h	Increase in CAT, GPx and GST activities	Ozcan Oruc and Uner, 2004
	Gill		Increase in SOD activity	
C. auratus	Liver	0.005 - 1.0 mg/l for 40 days	Alteration in CAT, SOD and GPx activities	Zhang et al., 2004

Table 3. Effects of 2,4-D on fish

2.2.3 Rats

On view of the data concerning rats, it can be concluded that exposure to 2,4-D induced oxidative stress and lipid Peroxidation (table 4). The first important study was done by Celik et al. (2006), who studied the effects of 2,4-D on serum marker enzymes, erythrocyte and tissue antioxidant defense and lipid peroxidation in rats. The authors found that the administration of 1.5 and 3mg/day of 2,4-D during 25 days induced in vivo oxidation. Recently, from the series of experiments described by Tayeb et al. (2010; 2011 b; 2011c) and

Nakbi et al. (2010; 2011a; 2011b): it is quite evident that subacute exposure of rats to 5, 15, 75 and 150 mg/kg BW during 28 days caused significant negative changes in the erythrocyte, liver and kidney functions. The fatty acid composition of the erythrocyte membranes also of hepatocytes has been altered by the 2,4-D exposure with 2,4-D exposure increased levels of SFA and the decreased level of unsaturated fatty acids (UFA); increase in the index of fatty acid unsaturation. These results may explain the higher amounts of MDA observed in 2,4-D treated group. Furthermore, the antioxidant enzyme activities, in liver, erythrocytes and kidneys were significantly affected.

Thus, results described in table 4 indicated the potential effects of 2,4-D to cause oxidative stress in rat. These results are partly in accordance despite the differences between studies in their settings, materials and experimental designs. While, in an investigation done by Dinamarca et al. (2007) who looked for the effects of 2,4-D on the generation of oxidative stress during early pregnancy in mice, they proved that 2,4-D in the concentrations usually found in blood can not provoke oxidative stress. Indeed, these negative results agree with others in 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 observations of oxidation being induced in rats.

3. The 2,4-D exposure and human diseases

Works done by Mountassif et al. (2008) Nakbi et al. (2011) and Tayeb et al., (2011 b; 2011c) clearly demonstrated that subacute exposure to 2,4-D significantly modified lipidic status, disrupt lipid metabolism, also, we have noted an increase in the LDL/HDL and TC/HDL ratios, which are pertinent indices of the incidence of cardiovascular risk. All these findings support the hypothesis that high doses of 2,4-D might contribute to development of vascular and cardiac pathologies.

Indeed, some pesticides have been implicated in the pathogenesis of cardiovascular disorders, hypertension and other health related problems (Singh et al., 2007). Kang et al. (2006) have noted that there are long-term health consequences of Agent orange herbicide (a mixture of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)) exposure among army Vietnam veterans who were exposed to this herbicide. The study group showed significantly higher risk of diabetes, heart diseases, and circulatory diseases such as hypertension. Also, England (1981) reported that prolonged exposure to herbicides such as 2,4-D has been associated with Coronary Artery Ectasia (CAE). It was reported that more than 50% of CAE were caused by atherosclerosis (Lin et al. 2008). Recently, Schreinemachers (2010) indicate that human exposure to 2,4-D was associated with changes in biomarkers that, have been linked to risk factors related to the pathogenesis of acute myocardial infarction and type-2 diabetes, such as dyslipidemia and impaired glucose metabolism.

Matrices	Doses	Results	References
- Erythrocytes, brain, liver, kidney, heart	1.5, 3mg/kg/B.W/day during 25 days	- Induction of in vivo oxidation: changes in the GSH, GST, GR, SOD, CAT activities and MDA levels.	Celik et al., 2006
- Liver	3 mg/kg/B.W/day for 4 weeks	- Moderated oxidative stress in liver cells: increase of lipide peroxidation (MDA) and decrease in CAT activity	Mountassif et al., 2008
- Blood	0.01, 0.1 and 100 mg/kg/B.W/day during gestation days 0–9	- Catalase activity and TBARs were not modified -TAC (Total antioxidant capacity) was significantly decreased at 100 mg/kg/d of 2,4-D	Dinamarca et al., 2007
- Erythrocytes	5 mg/kg/B.W/day for 4 weeks	- Significant decrease of SOD, GPx, GR and CAT activities - Changes of the fatty acid profile in erythrocyte membranes	Nakbi et al., 2010 a
- Liver	5 mg/kg/B.W/day for 4 weeks	- Increased hepatic lipid peroxidation (MDA, conjugated dienes) and decreased hepatic antioxidant enzyme activities (SOD, CAT, GPx, GR) - Modification of liver's fatty acid composition	Nakbi et al., 2010 b
- Erythrocytes	15, 75 and 150 mg/kg/B.W/day for 4 weeks	- The MDA level was significantly increased in 2,4-D treated groups. - Fatty acid composition of the erythrocytes was also significantly changed with 2,4-D exposure, in favor of the peroxidation of polyunsaturated fatty acids. - Antioxidant enzyme (SOD, CAT, GPx, and GR) activities were significantly decreased	Tayeb et al., 2011 b
- Liver	15, 75 and 150 mg/kg/B.W/day for 4 weeks	- Significantly increase in The MDA and conjugated dienes level - Fatty acid composition of the liver was significantly changed - Hepatic antioxidant enzyme (SOD, CAT, GPx, and GR) activities were significantly affected.	Tayeb et al., 2011c Tayeb et al. 2010
- Kidney	15, 75 and 150 mg/kg/B.W/day for 4 weeks	- Increase in kidney MDA - The activities of CAT, SOD, GPx, GR were significantly affected due to 2,4-D exposure.	Tayeb et al., 2011 c
- Kidney	600 mg/L from the 14th day of pregnancy until day 14 after delivery.	- Increase in TBARs and protein carbonyl levels - Decrease in antioxidant enzyme activities (CAT, SOD, GPx) in the kidneys of suckling pups and their mothers. - Significant decline in kidney glutathione, non-protein thiol and vitamin C levels.	Troudi et al., 2011

Table 4. Effects of 2,4-D on rats

4. Protective effects of some antioxidants on 2,4-D toxicity

Several scavenging agents and antagonists are established to reduce pesticides toxicity (Kalender et al., 2004; Grajeda-Cota et al., 2004). The prevention of the peroxidation processes by using antioxidants and free radical sweepers of plant origin becomes an important issue of clinical nature. So, in their *in vitro* investigations, Bors et al. (2009; 2011) have evaluated the impact of extracts of *Uncaria tomentosa* leaves and bark on human erythrocytes as well as the antioxidant properties of U. *tomentosa* extracts against oxidative stress induced by 2,4-D and its environmental transformation products 2,4-DCP and catechol. Their studies showed that U. *tomentosa* extracts protected against the induction of hemolysis, haemoglobin oxidation and ROS increase in human erythrocytes incubated with 2,4-D (Bors et al., 2009).

Other in vivo studies have been investigated in the exploitation of the anti-inflammatory and vascular protective properties of olive oil polyphenols. Recently, Nakbi et al. (2010a; 2010b; 2011) confirmed the beneficial effects of extra virgin olive oil and its hydrophilic and lipophilic fractions for their lipid-lowering, antioxidative, and protective effects against oxidative damage induced by 2,4-D. In fact, extra virgin olive oil and its extracts administered to 2,4-D-treated rats protected tissues and erythrocyte membranes against oxidative damage by means of preventing excessive lipid peroxidation to increase the monounsaturated fatty acid composition and by maintaining serum marker enzymes and antioxidants enzymes at near normal concentrations. So including olive oil in the diet may offer benefits in decreasing tissue damage and the atherosclerotic process during 2,4-D exposure in rats.

5. Conclusions

Until nowadays, acute toxicological tests have been conducted on the various forms of 2,4-D. The precise mechanism of 2,4-D acute toxicity may involve disruption of plasma and intracellular membranes or uncoupling of oxidative phosphorylation; this last mechanism was involved in the generation of oxidative stress. In fact, Several lines of evidence indicate that oxidative stress and ROS formed in the presence of 2,4-D could be responsible for its toxic effects in many settings *in vitro* and *in vivo*. Consequently, increased tissue oxidative stress can lead to cell damage. Given the implications of oxidative stress in several human genetic diseases, ageing, inflammation and cancer development, these results are of concern in situations of eventual massive or repeated exposure to the herbicide. In recent years, scientists have focused on the preventive effects of some natural antioxidant against degenerative diseases mediated by the ROS. 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. Further experimental evidence, mechanism-oriented studies and clinical trials are needed to understand and to further characterize the toxic effects of 2,4-D herbicide.

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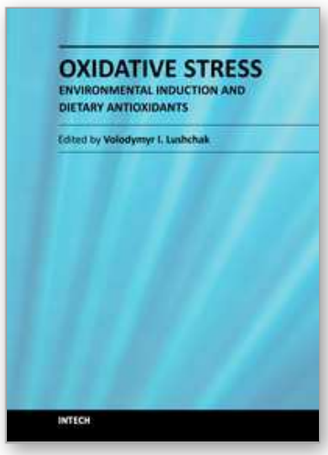

Oxidative Stress - Environmental Induction and Dietary Antioxidants

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This book focuses on the numerous applications of oxidative stress theory in effects of environmental factors on biological systems. The topics reviewed cover induction of oxidative stress by physical, chemical, and biological factors in humans, animals, plants and fungi. The physical factors include temperature, light and exercise. Chemical induction is related to metal ions and pesticides, whereas the biological one highlights host-pathogen interaction and stress effects on secretory systems. Antioxidants, represented by a large range of individual compounds and their mixtures of natural origin and those chemically synthesized to prevent or fix negative effects of reactive species are also described in the book. This volume will be a useful source of information on induction and effects of oxidative stress on living organisms for graduate and postgraduate students, researchers, physicians, and environmentalists.

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