Journal of Animal and Veterinary Advances 7 (11): 1480-1488, 2008
ISSN: 1680-5593

The Effects of the Dose-dependent γ-hexachlorocyclohexane (Lindane) on Blood and Tissue Antioxidant Defense Systems, Lipid Peroxidation and Histopathological Changes in Rats

[1]A. Fatih Fidan, [3]I. Hakkı Cigerci, [1]Nalan Baysu-Sozbilir, [1]Ismail Kucukkurt,
[2]Hayati Yuksel and [2]Hikmet Keles
[1]Department of Biochemistry, [2]Department of Pathology,
Faculty of Veterinary Medicine, University of Afyon Kocatepe,
03200 Afyonkarahisar, Turkey
[2]Department of Biology, Faculty of Science and Literature,
University of Afyon Kocatepe, 03200 Afyonkarahisar, Turkey

Abstract: In the present study, we have sought the effects of lindane on antioxidant parameters and nitric oxide (NOx) levels of blood, liver, kidney and brain, as well as its histopathological evaluation in rats. The rats were divided into four groups each containing 10 rats: control; L10, L20 and L40. C group was administered by 1 mL day^{-1} pure olive oil. The other groups, L10, L20 and L40 were administrered by 10, 20 and 40 mg/kg/bw orally lindane, respectively for 4 weeks. Administration of lindane caused an increase in malondialdehyde (MDA), total antioxidan activities (AOA) and NOx concentrations in blood ($p<0.05$), but 10 mg kg^{-1} dosage of lindane treatment did not cause any difference in blood and tissue MDA levels. Moreover, MDA levels in the liver, kidney and brain increased ($p<0.05$) at 20 and 40 mg kg^{-1} dosage of lindane treatment. The liver, kidney and brain reduced glutathione (GSH) concentrations decreased in all lindane groups ($p<0.05$). An increase in the kidney NOx concentrations was observed in lindane treated animals ($p<0.05$). However, liver NOx levels were increased only L40 group ($p<0.05$). Brain GSH concentrations between groups did not differ. Histopathologically, severe liver and kidney congestion were detected in lindan groups, but no specific changes was seen in the brain. While no significant histopathological changes were observed in the tissues of the animals in L10 group, megalocytosis in hepatocytes, periacinar settled parancimateus and vacuolar degeneration as well as sinusoidal and venous c ongetion and also periportal lymphocytic infiltrations were observed in liver of L20 and L40 groups. Medullar and cortical haemorrhagie, degeneration and vacuolisations of proximal convoluted tubules were seen in kidney. Furthermore, hyperemie was seen in the parancimateus brain vessels.

Key words: Lindane, oxidative stress, tissue, NOx, histopathological changes

INTRODUCTION

Pesticides are agents used to kill or control undesired pests, such as insects, weeds, rodents, fungi, bacteria or other organisms. Pesticides have a significant public health benefit by decreasing the food borne and vector borne diseases (Ecobichon, 1996). However, these health benefits are not achieved without potential risk and possible adverse health effects to humans, domesticated animals and the environment (Repetto and Baliga,1996).

Lindane (hexachlorocyclohexane; gamma HCH; gamma-BHC) is an organochlorine insecticide and as a therapeutic scabicide, pediculicide and ectoparasiticide or humans and animals. However, like many of the organochlorine insecticides, lindane's persistence in the environment and its biomagnification potential (Safe, 1993; Budavari *et al*., 1989).

Normal cellular function depends on a balance between reactive oxygen species produced and antioxidant defense mechanisms available to the cell. Reactive Oxygen Species (ROS) arise as by-products of normal cellular metabolism or may be the consequence of exposure to certain chemicals (Kerr *et al*., 1996; Moslen, 1994; Krieger and Caruso, 2001). Reactive species derived from chemicals, oxygen, or nitrogen have been implicated as putative noxious intermediates responsible for cellular

Corresponding Author: A. Fatih Fidan, Department of Biochemistry, Faculty of Veterinary Medicine, University of Afyon Kocatepe, 03200, ANS Campus, Afyonkarahisar, Turkey

damage. Because electrophilic metabolites or radicals and excited species can readily interact with essential biomolecules, covalent binding to cellular components or their oxidative modification can occur, leading to structural and functional alterations (Fernández *et al*., 2003; Comporti, 1989; Kappus, 1987).

Under normal condition, excessive formation of ROS and concomitant damage at cellular and tissue concentrations is controlled by cellular defense systems. These preventive defense systems can be accomplished by enzymatic and non-enzymatic antioxidant (Jalili *et al*., 2007). In instances in which the existing enzymatic and nonenzymatic cellular antioxidants are unable to counteract the ROS, a condition known as oxidative stress occurs. In such cases of overwhelmed antioxidant defense, cellular function can be affected and cells may be damaged (Krieger and Caruso, 2001).

Pesticide chemicals may induce oxidative stress leading to generation of free radicals and alterations in antioxidant and scavengers of oxygen free radicals (Banerjee *et al*., 1999). Lindane has been reported to induce oxidative stres, membrane perturbation, functional impairment in blood brain barrier, disturbance in glutathione homeostatis and alteration in cyt P450 monooxygenase enzymes. Lindane enchances oxidative stress by interacting with the cell membrane, triggering the generation of Reactive Oxygen Species (ROS) and altering the levels of antioxidant molecules and enzymes. Also, the modulation of antioxidant molecules clearly suggests that lindane is a potent inducer of oxidative stress and can cause severe physiological dysfunction in various organ systems (Banerjee *et al*., 1999, Barros *et al*., 1991; Bano and Bhatt, 2007).

The aim of the present study is to evaluate the effect of lindane on antioxidant defense systems, lipid peroxidation and NOx levels of blood and various tissues of rats, by histopathological examination and biochemical analysis and to elucidate the mechanism involved in this effect.

MATERIALS AND METHODS

Chemicals: Lindane (CAS number: 58-89-9) and other chemicals used in the study were purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co. St. Louis, MO, USA).

Animals and experimental design: Male Sprague-Dawley rats, weighing about 250-280 g were used. They were housed under standard conditions of temperature (23±2°C), humidity and dark-light cycle (lights on from 6:00 am to 6:00 pm). The animals were maintained on standard rat feed supplied by Bil- Yem Ltd. (Turkey). Tap water was available ad libitum. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the University of Afyonkarahisar Kocatepe Animal Ethics Committee. Experimental animals were divided into 4 groups each containing 10 rats each: group I (control) 1 mL day^{-1} pure olive oil, group II (L10) 10 mg/kg/bw-lindane, group III (L20) 20 mg/kg/bw-lindane and group IV (L40) 40 mg/kg/bw-lindane. Lindane was prepared freshly each day. The doses of 10, 20, 40 mg/kg/bw were dissolved in 1 mL olive oil and given daily to the relevant groups bygastric gavage for 30 days. The control group was given only olive oil at the same volume. The clinical findings observed in the rats were recorded during the experimental period.

Biochemical estimation: At the end of the experimental period, the rats were anaesthetized and killed by cervical dislocation. Blood samples and organs including brain, liver and kidney were collected for examination of clinical biochemistry. Blood samples were taken into heparinized tubes in all subjects from heart. One milliliters of blood were immediately pipetted into another tube to measure MDA. Remaining blood was centrifuged for plasma separation. Plasma samples were stored at -30°C for the analysis of AOA and NOx. The tissues were collected for examination of clinical biochemistry, removed immediately and washed in ice-cold saline. Tissues were homogenized 1.40 w v^{-1} in 0.1M phosphate buffer, pH 7.4, containing 1 mM EDTA. After centrifugation at 18 000×g for 15 min at 4°C, the supernatant was extracted and kept at -30°C in advance of assays. In the tissue homogenates the levels of MDA, GSH and NOx were assayed. The vital organs (liver, kidney and brain) of each rat were carefully removed. Pieces of this organs were fixed in 10% neutral formol saline for further histopathological investigations.

Determination of blood and tissue malondialdehyde levels: MDA levels, an index of lipid peroxidation, were measured by the double heating method of Draper and Hadley (1990). The method is based on spectrophotometric measurement of the purple color generated by the reaction of TBA with MDA. For this purpose, 2.5 mL of trichloroacetic acid solution (10%, w v^{-1}) was added to 0.5 mL whole blood in each centrifuge tube and tubes were placed in a boiling water bath for 15 min. After cooling to room temperature, the tubes were centrifuged at 1000 g for 10 min and 2 mL of each sample supernatant was transferred to a test tube containing 1 mL of TBA solution (0.67%, w v^{-1}). Each tube was then placed in a boiling water bath for 15 min. After cooling to room

temperature, the absorbance was measured at 532 nm by using the Shimadzu UV 1601 spectrophotometer. The concentration of MDA was calculated based on the absorbance coefficient of the TBA-MDA complex ($\varepsilon = 1.56\times105\ cm^{-1}\ M^{-1}$). The levels of MDA in tissues was determined according to the method described by Okhawa *et al.* (1979). In this method, MDA reacts with thiobarbituric acid to form a colored complex that has maximum absorbance at 532 nm. MDA levels as nmol/g wet tissue was expressed.

Estimation of plasma and tissue nitric oxide levels: Nitric oxide decomposes rapidly in aerated solutions to form stable nitrite/nitrate products (Nox). Plasma and tissues nitrite/nitrate concentration was measured by a modified method of Griess assay, described by Miranda *et al.* (2001). The principle of this assay is reduction of nitrate by v anadium combined with detection by the acidic Griess reaction. Briefly, samples were deproteinized prior to assay. The supernatants (0.5 mL) 0.25 mL of 0.3 M NaOH were added. After incubation for 5 min at room temperature, 0.25 mL of 10 % (w v^{-1}) $ZnSO_4$ was added for deproteinization. This mixture was then centrifuged at 14000×g for 5 min and supernatants were used for the Griess assay. The plasma was added to 96% cold ethanol at 1.2 (v v^{-1}) and then vortexed for 5 min. After incubating for 30 min at 4°C, the mixture was centrifuged at 8000 g for 5 min and the supernatants were used for the Griess assay. Total 100 μL of filtrated plasma was mixed with 100 μL of Vcl 3 and was rapidly followed by the addition of the Griess reagents, which are containing SULF 50 μL and NEDD 50 μL. The determination was performed at 37°C for 30 min. The absorbance was measured by a microplate reader (Multiskan Spectrum, Thermo Labsystems, Finland) at 540 nm. Nitrite/nitrate concentration was calculated using a $NaNO_2$ standard curve and expressed as μM/L.

Determination of tissue reduced glutathione levels: The tissues GSH concentration was measured using the method described by Beutler *et al.* (1963). Briefly, 0.2 mL supernatant was added to 1.8 mL distilled water. Precipitating solution of 3 mL (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 no. 42). Filtrate of 2 mL was taken and added into another tube and then 8 mL of the phosphate solution (0.3 M disodium hydrogen phosphate) and 1 mL DTNB were added. A blank was prepared with 8 mL of phosphate solution; 2 mL diluted precipitating solution (3-2 parts distilled water) and 1 mL DTNB reagent. A standard solution of the GSH was prepared (40 mg/100 mL). The optical density was measured at 412 nm in the spectrophotometer. Results were communicated as mg/g wet tissue.

Determination of plasma total antioxidant activity: The total AOA was determined using the method described by Koracevic *et al.* (2001). The assay measures the capacity of the serum to inhibit the production of TBA reactive substances (TBARS) from sodium benzoate, under the influence of the oxygen free radicals derived from Fenton's reaction. The reaction was measured spectrophotometrically at 532 nm. Antioxidants from the added sample cause suppression of the production of TBARS and the inhibition of color development is defined as AOA. A solution of 1 mmol L^{-1} uric acid was used as standard.

Histopathological estimation: After blood sampling for the biochemical analysis, the animals were sacrificed, quickly necropsied and small piecess of liver, kidneys and brain were taken for histopathological examination. Organ samples were fixed in 10% neutral formol saline, embedded in paraffin and cut on a microtome in 4-5 μ thick and stained with hematoxylin-eosine (Allen, 1992) then examined under light microscopy.

In present study, lesions were given the histopathological picture depending on the severity of changes.

Statistical analysis: All data were presented as mean±S.E. for parametric variables. Parametric variables were compared using one-way analysis of variance with post-hoc analysis using the Duncan test. Data were analyzed using the SPSS® for Windows computing program (Version 10.0) and $p<0.05$, was considered statistically significant (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

The results of blood MDA, AOA and NOx levels in controls and other experimental groups were summarised in Table 1.

Lindane treatment (20 and 40 mg/kg/bw lindane) significantly increased the blood MDA levels as compared to control and L10 groups ($p<0.05$). Application

Table 1: Effects of lindane on MDA, AOA and NOx content in blood of rats

Parameters	Control $\bar{X}$ ±S.E	L10 $\bar{X}$ ±S.E	L20 $\bar{X}$ ±S.E	L40 $\bar{X}$ ±S.E
MDA (nmol mL^{-1})	$2.27\pm0.27^{b*}$	$02.48\pm0.48^{b*}$	$03.36\pm0.46^{ab*}$	$04.39\pm0.36^{a*}$
AOA (mM)	$4.57\pm0.29^{a*}$	$03.52\pm0.39^{b*}$	$03.48\pm0.22^{b*}$	$03.20\pm0.30^{b*}$
NOx (μmol L^{-1})	$9.65\pm0.88^{b*}$	$12.00\pm0.45^{ab*}$	$12.62\pm0.95^{ab*}$	$13.76\pm1.09^{a*}$

Values are shown as mean±S.E. Values with different letters show statistically significant differences (*: $p<0.05$)

Table 2: Effects of Lindane on MDA, GSH and NOx content in various tissues of rats

Tissue	Parameters	Control $\bar{X}$ ±S.E	L10 $\bar{X}$ ±S.E	L20 $\bar{X}$ ±S.E	L40 $\bar{X}$ ±S.E
Liver	MDA (nmol g^{-1})	02.99±0.39^{b*}	03.46±0.3^{b*}	04.31±0.59^{ab*}	05.46±0.79^{a*}
	GSH (mg g^{-1})	15.91±0.62^{a*}	14.26±0.33^{b*}	13.38±0.42^{bc*}	12.75±0.44^{c*}
	NOx (µmol L^{-1})	19.92±2.89^{b*}	20.24±2.13^{b*}	20.13±4.20^{b*}	31.24±2.46^{a*}
Kidney	MDA (nmol g^{-1})	05.40±0.27^{c*}	05.93±0.08^{bc*}	07.23±0.53^{ab*}	07.73±0.87^{a*}
	GSH (mg g^{-1})	15.15±0.58^{a*}	13.79±0.23^{b*}	12.71±0.34^{b*}	12.59±0.49^{b*}
	NOx (µmol L^{-1})	09.89±2.51^{b*}	13.70±2.61^{ab*}	15.15±1.12^{a*}	15.21±1.25^{a*}
Brain	MDA (nmol g^{-1})	04.98±0.14^{b*}	06.24±0.40^{b*}	07.79±0.45^{a*}	07.97±0.42^{a*}
	GSH (mg g^{-1})	18.86±0.25^{a*}	15.46±0.47^{b*}	15.75±0.62^{b*}	14.33±0.40^{b*}
	NOx (µmol L^{-1})	13.24±0.96	14.19±1.83	14.9±1.32	15.15±1.85

Values are shown as mean±S.E. Values with different letters show statistically significant differences (*: $p<0.05$)

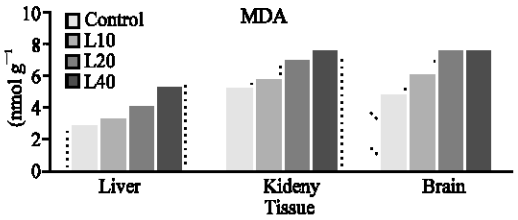

Fig. 1: The marker of lipid peroxidation MDA levels in liver, kidney and brain of the rats significantly increased ($p<0.05$) in L20 and L40 groups compared to control group. Whereas liver, kidney and brain MDA levels of L10 group didn't show any significant change (Table 2 and Fig. 1)

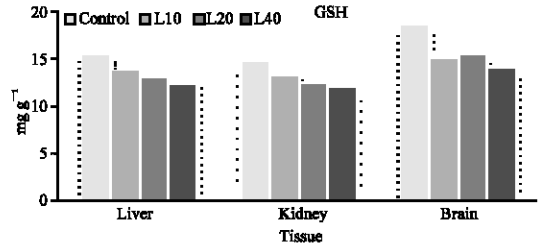

Fig. 2: The liver, kidney and brain GSH concentrations significantly decreased in all lindane-treated group compared to control group ($p<0.05$). It was observed that lindane application causes a significant decrease in tissue GSH (Table 2 and Fig. 2)

of 10 mg/kg/bw-lindane did not cause any significant difference in MDA levels. So that 20 and 40 mg/kg/bw lindane treatment was associated with significant lipid peroxidation. The mean AOA following lindane application was significantly lower compared to control groups ($p<0.05$). Thus, it was observed that lindane application causes a significant decrease in AOA. Mean plasma NOx concentrations was significantly higher in the lindane treatment groups in contrast to the control animals ($p<0.05$). However, NOx level was higher in L40 group as compared to the 10 and L20 groups.

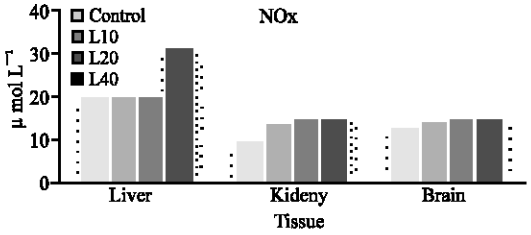

Fig. 3: The liver NOx levels were significantly increased in the L40 group compared to control group and other experimental groups ($p<0.05$). Mean kidney NOx concentrations was significantly higher in the lindane treatment groups in contrast to the control animals ($p<0.05$). However, NOx level was higher in 20 and L40 group as compared to the L10 and control groups. Brain NOx concentrations between groups did not differ (Table 2 and Fig. 3)

The results of all determination for the experimental and the control group tissues are given in Table 2 and Fig. 1-3.

Histopathological changes in organs of experimental groups vary from different doses of lindane (10, 20, 40 mg/kg bw daily during a 4 week period) were described. No macroscopic or microscopical alterations were observed in the liver, kidney and brain of the control rats. Severe liver and kidney congestion were detected in lindan groups, but no specific changes was seen in the brain. While no significant histopathological changes were observed in the liver, kidney and brain of the animals in L10 group, megalocytosis in hepatocytes, periacinar settled parancimateus and vacuolar degeneration as well as sinusoidal and venous congetion and also periportal lymphocytic infiltrations were observed in liver of L20 and L40 groups (Fig. 4). In the kidney, medullar and cortical haemorrhagie, degeneration and vacuolisations of proximal convoluted tubules were seen (Fig. 5). Furthermore, hyperemie was seen in the parancimateus brain vessels.

The interest in ROS in biology and medicine has been increased because of their strong relationship

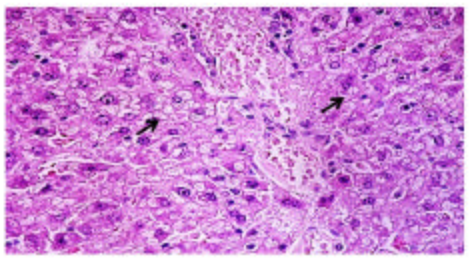

Fig. 4: Periacinar settled parancimateus andvacuolar degeneration (arrows), HE×400

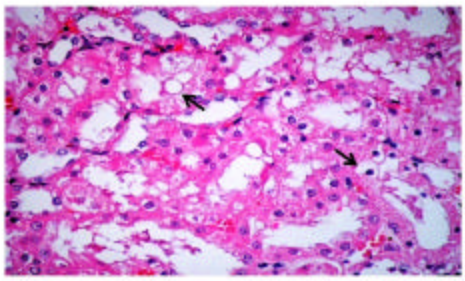

Fig. 5: Degeneration and vacuolisations of proximal convoluted tubules (arrows), HE×400

with phenomena such as aging and disease processes (Cao *et al*., 1995). ROS are produced as a normal product of cellular metabolism. The continual formation of ROS and other free radicals is important for normal physiological functions and cellular redox reactions under normal conditions. However, excessive generation of free radicals can occur due to endogenous biological or exogenous environmental factors, such as exposure to radiation, pollution or chemical substances (Misra and Fridovich, 1972). A cell defends itself against ROS by elaborating systems of biological defence. In spite of numerous biological defense system, increased free radical generation has the potential to result in oxidative stress. Oxidative stress may result from an imbalance between ROS and antioxidants levels (Lightboy *et al*., 2001). It is well known that, when the organism cannot balance free radical generation with the defense systems, a cellular injury and tissue damage migh occur. The main damage induced by ROS results in alterations of cellular macromolecules (membrane lipids, proteins and DNA) and changes in intracellular calcium and intracellular pH, or cell death (Dorval *et al*., 2003; Fidan and Dundar, 2008).

Pesticides may induce oxidative stress leading to generation of free radicals and alteration in antioxidant or oxygen free radical scavenging enzyme system. One ofthe reason for pesticide toxicity in the production of ROS may be due to their redox-cycling activity-they readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and hence hydrogen peroxide through dismutation reaction. The other could be due to generation of free radicals which might depend on the alteration in the normal homeostasis of the body resulting in oxidative stress (Banerjee *et al*., 1999; Jalili *et al*., 2007). As well as lindane is an organochlorine insecticide, induces oxidative stress by interacting with the cell membrane, triggering the generation of ROS and other free radical intermediates and altering the levels of antioxidant molecules or enzymes (Banerjee *et al*., 1999; Barros *et al*., 1991; Bano and Bhatt, 2007). Moreover, degeneration of kidney tubules, liver damage associated with fatty tissue and hystoplastic anemia have been observed in individuals poisoned by lindane (Manahan, 2003).

The impact made by free radicals on lipids is named as Lipid Peroxidation (LP). LP is a complicated radical chain reaction leading to the formation of various products including lipid hydroperoxides, conjugated dienes and malondialdehyde. Detection of lipid hydroperoxides and conjugated dienes and thiobarbituric acid-reactive substances (TBARS) such as MDA, are often applied to the study of lipid peroxidation reactions (Diplock, 1994; Enginar *et al*., 2006). Since, membrane phospholipids are major targets of oxidative damage, lipid peroxidation is often the first parameter analyzed for proving the involvement of free radical damage. Thus, the presence of MDA is considered as an indicator of free-radical damage through membrane lipid peroxidation (Katz *et al*., 1996; Enginar *et al*., 2006).

The present study reports that oral administration of lindane cause on oxidative stress in blood, liver, kidney and brain in rats. As reported by other authors, LP is one of the main manifestations of oxidative damage in cells and tissues. LP produces a progressive loss of cell membrane integity, imparement in membrane transport function and disruption of cellular ion homeostasis (Bano and Bhatt, 2007). Junqueira *et al*. (1986) demonstrated that TBARS increased in the liver homogenates after 24 h following 20 or 80 mg/kg ip lindane exposure in a dose dependent manner with increasing concentration of lindane. Moreover, Junqueira *et al*. (1997), conducted another study to examine oxidative stress related parameters following a 60 mg kg^{-1} dosing in rats. Again, the same trends as noted above were observed. Bagchi and Stohs (1993) examined in vitro lindane (0-200 ng mL^{-1}) for 30 min in rats in peritoneal macrophages and hepatic microsomal and mitochondrial fractions. They observed an increase in chemiluminescence in all samples indicating the generation of free radical species as a result of lindane exposure. Videla *et al*. (1990) and Videla (2000) indicate

that lindane enhance the expression and activity of cytP4502E1 in rat liver. These effects occur regardless of the changes in total cytP450 content, involve a substantial increase in the molecular activity of NADPH-cytP450 reductase of liver microsomes and may represent contributory mechanisms for NADPH-dependent O_2 generation and related hepatotoxicity. The present study shows that blood, liver, kidney and brain MDA levels of L10 group did not differ, but MDA increased significantly in the liver, kidney, brain and blood after 20 or 40 mg kg^{-1} lindane exposure, in agreement with the previous studies. In addition our results showed that lipid peroxidation did not occur at low dose (10 mg kg^{-1}) of lindane exposure.

The antioxidant defense system includes small molecular antioxidants, antioxidant enzymes and metal chelating agents. Halliwell and Gutteridge (1998) define an antioxidant as any substance that when present at low concentrations, compared to those of an oxidizable substrate, significantly delays, or inhibits, oxidation of that substrate. The efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially increase due to an induction but later enzyme depletion results, resulting in oxidative cell damage (Vidyasagar *et al*., 2004). The total AOA of body fluids expresses a cooperative interaction between various antioxidants and is crucial for the maximum suppression of a free radical reaction in extracellular compartments (Cizova *et al*., 2004). Antioxidant activity indicates the antioxidant characteristics of only one antioxidant, whereas total antioxidant activity represents the total antioxidant characteristics of all antioxidants found in the plasma. Enzymatic scavengers like SOD, CAT, glutathione peroxidase (GPx), Glutathione Reductase (GR) etc. protect the system from deleterious effects of reactive oxygen species and pesticides have been reported to cause alteration in antioxidants or free radical scavenging system (Oberoi *et al*., 2007) In addition to AOA, reduced GSH and its metabolizing enzymes provide the major defense against ROS-induced cellular damage (Celik and Suzek, 2008). GSH serves as a reductant in oxidation reactions resulting in the formation of GSSG. GSH can protect cells against the damage of ROS and free radicals that arise during conditions of oxidative stress (Loch-Caruso *et al*., 2005). Thereby decreased GSH levels may reflect depletion of the antioxidant reserve. As a consequence of GSH deficiency, a number of related functions may be impaired such as a decrease in reducing capacity, protein biosynthesis, immune function, accumulations of lipid peroxidation products and detoxification capacity (Annuk *et al*., 2001; Sen, 2000; Hayes and McLellan, 1999). In the present study the AOA levels following lindane treatment decreased compared to control groups ($p<0.05$). Thus, it was observed that lindane application causes a significant decrease in AOA. However, Barros *et al*. (1991) reported that 20 ppm dietary lindane suplementation in to diets of rats for 15 or 30 days caused an oxidative stres by increasing levels of, cytP450, TBARS in liver homogenates and superoxide. Lindane induces oxidative stress by involving the activity of cyt P450 system which results into the generation of superoxide radicals. Increased generation of superoxide radicals lead to oxidation and depletion of GSH with a lipid peroxidative response (Bano and Bhatt, 2007). The results in the present study also showed that, GSH concentrations significantly decreased in liver, kidney and brain by lindane intoxication, paralellto the previous studies in rat liver. In this context, it is possible that the observed insufficiency in antioxidant activity could be due to direct modification of the antioxidant defenses by lindane.

Nitric oxide, a magic free radical gas molecule, has been shown to be involved in numerous physiological and pathophysiological processes. Endogenous NO is produced almost exclusively by L-arginine catabolism to L-citrulline in a reaction catalyzed by a family of nitric oxide synthases. In the first step, Arg is hydroxylated to an enzyme-bound intermediate Nù-hydroxy-l-arginine and 1 mol of NADPH and O_2 are consumed. In the second step, NHA is oxidized to citrulline and NO, with consumption of 0.5 mol of NADPH and 1 mol of O_2. Oxygen activation in both steps is carried out by the enzyme-bound heme, which derives electrons from NADPH (Wang *et al*., 2005.). Nitric oxide is a endothelium-derivated relaxing factor as a signaling molecule in the normal physiology of mammalian (Boeckxstaens *et al*., 1991). The role of NO seems to be controversial, because it has been shown that tissue dysfunction or injury could occur after inhibition of NO. However, high production of NO has been suggested as a cause of tissue injury (Bohloli *et al*., 2007). Stimulation of tissue production of 'NO is also associated with adverse events such as hypotension, inhibition of intermediary metabolism and the production of the potent oxidant peroxynitrite ($ONOO^-$) following radical-radical reaction with superoxide (Rubbo *et al*., 1994). The bioavailability of NO is reduced due to the increased level of superoxide radical, which transforms NO to peroxynitrite (Žourek *et al*., 2008). Fernández *et al*. (2003) reported that the activity of the NADPH-generating enzyme glucose-6-phosphate dehydrogenase was not altered by lindane, but NADPH is required for nitric oxide synthesis (Žourek *et al*., 2008).

In the present study contrary to the reported resuls of the previous sttudies plasma NOx concentrations was

significantly higher in 20 and 40 mg kg^{-1} lindane treated groups. Similarly significant increase in the kidney NOx concentrations was observed in lindane treated animals. NOx levels in liver increased only 40 mg kg^{-1} dosage of lindane treatment. Furthermore brain NOx concentrations between groups did not differ. Depending on the acquired results, new studies should be carried outto understand this mechanism in details.

High doses of lindane cause liver hypertrophy, kidney tubular degeneration, hyaline droplets, tubular distension, interstitial nephritis and basophilic tubules in rats. Suter (1983) observed liver and kidney effects, centrilobular hypertrophy and necrosis, tubular distension and basophilic tubules, respectively in rats were fed lindane. Videla *et al*. (1995) reported extensive liver necrosis and the presence of granulomas containing lymphocytes, kupffer cells and polymorphonuclear leukocytes. Similar microscopical findings were observed suitable to previous studies in the present study. Histopathological changes in organs of experimental groups vary from different doses of lindane (10, 20, 40 mg kg^{-1} bw daily during a 4 week period) were described. In the experimental groups, no significant histopathological changes were observed in the liver, kidney and brain of animals in the L10 group by microscopic observations, but, megalocytosis in hepatocytes, periacinar settled parancimateus and vacuolar degeneration as well as sinusoidal and venous congetion and also periportal lymphocytic infiltrations were observed in liver of 20 and L40 groups. Medullar and cortical haemorrhagie, degeneration and vacuolisations of proximal convoluted tubules were evaluated in the kidney. Furthermore, parancimateus vessels hyperemie was seen in the brain.

CONCLUSION

It was concluded that, lindane induced oxidative stress in blood and tissue by decreasing the activities of antioxidant enzymes and generation of free radicals in rats. Therefore, it was thought that oxidative stress both in blood and tissue increased in a dose dependent manner paralel to the increase in administrated dose of lindane. Moreover, the clinical trials of the antioxidants could provide great advantages to patients suffering from lindane toxications and maintain life span of animals and humans. On the other hand, it was beliaved further studies should be carried out to deterimine the relationship between lindane and NOx.

REFERENCES

Allen, T.C., 1992. Hematoxylin and eosin. Laboratory methods in histotechnology. In: Prophet, E.B., B. Mills, J.B. Arrington and L.H. Sobin (Eds.). 1st Edn. American Registry of Pathology Washington, pp: 53-58. ISBN: 978-1881041009.

Annuk, M., M. Zilmer, L. Lind, T. Linde and B. Fellström, 2001. Oxidative stress and endothelial function in chronic renal failure. J. Am. Soc. Nephrol., 12: 2747-52. PMID: 11729244.

Bagchi, M. and S.J. Stohs, 1993. *In vitro* Induction of Reactive Oxygen Species by 2, 3, 7, 8 Tetrachlorodibenzo-P-dioxin, Endrin and Lindane in Rat Peritoneal Macrophages and Hepatic Mitochondria and Microsomes. Free Radic. Biol. Med., 14 (1): 11-8. PMID: 7681024.

Banerjee, B.D., V. Seth and A. Bhattacharya, 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol. Lett., 107 (1-3): 33-47. PMID: 10414779.

Bano, M. and D.K. Bhatt, 2007. Neuroprotective role of a novel combination of certain antioxidants on lindane (g-HCH) induced toxicity in cerebrum of mice. Res. J. Agric. Biol. Sci., ANSInet Pub., 3 (6): 664-669.

Barros, S.B.M., K. Simizu and V.B.C. Junqueira, 1991. Liver Lipid Peroxidation-Related Parameters after Short-term Administration of Hexachlorocyclohexane Isomers to Rats. Toxicol. Lett., 56 (1-2): 137-44. PMID: 1708174.

Beutler, E., O.B. Dubon and M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888. PMID: 423305.

Boeckxstaens, G.E., P.A. Pelckmans, J.J. Bogers, H. Bult, J.G. De Man, L. Oosterbosch, A.G. Herman and Y.M. Van Maercke, 1991. Release of nitric oxide upon stimulation of nonadrenergic noncholinergic nerves in the rat gastric fundus. J. Pharmacol. Exp. Ther., 256: 441-447. PMID: 1671594.

Bohloli, M., H. Uzun, E. Aytac, A.S. Toklu, M. Paksoy, H. Durak and T. Ipek, 2007. Hyperbaric Oxygen (HBO) Therapy after Partial Hepatectomy: An Experimental Study on Oxidative Stress in Rats. Scandinavian J. Lab. Anim. Sci., 34 (2): 131-140.

Budavari, S., M.J. O'Neil, A. Smith and P.E. Heckelman, 1989. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals. 11th Edn. S. Budavari. Merck and Co. Inc., Rahway, N.J., pp: 866-867. ISBN: 911910-28-X.

Cao, G., C.P. Verdon, A.B.H. Wu, H. Wang and R.L. Prior, 1995. Automated assay of oxygen radical absorbance capacity with the COBAS FARA II. Clin. Chem. 41: 1738-1744. PMID: 7497614.

Celik, I. and H. Suzek, 2008. Effects of subacute exposure of dichlorvos at sublethal dosages on erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. Ecotoxicol. Environ. Saf. PMID: 18539328.

Cizova, H., A. Lojek, L. Kubala and M. Ciz, 2004. The effect of intestinal ischemia duration on changes in plasma antioxidant defense status in rats. Physiol. Res., 53 (5): 523-531. PMID: 15479131.

Comporti, M., 1989. Three models of free radicalmediated cell injury. Chem. Biol. Interactions., 72: 1-56. DOI: 10.1016/0009-2797(89)90016-1.

Diplock, A.T., 1994. Antioxidants and disease prevention. Mol. Aspects Med., 15: 293-376. DOI: 10.1016/0098-2997(94)90005-1.

Dorval, J., V.S. Leblond and A. Hontela, 2003. Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (Oncorhynchusmykiss) exposed *in vitro* to endosulfan, an organochlorine pesticide. Aquat. Toxicol., 63: 229-241. PMID: 2711413.

Draper, H.H. and M. Hadley, 1990. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol., 186: 421-431. PMID: 2233309.

Ecobichon, D.G., 1996. Toxic effects of pesticides. Casarett and Doull's Toxicology the Basic Science of Poisons. In: Kalaassen, C.D. (Ed.). New York: Mc Grawhill, pp: 643-689. ISBN: 0071347216.

Enginar, H., G. Avci, A. Eryavuz, E. Kaya, I. Kucukkurt and A.F. Fidan, 2006. Effect of Yucca schidigera extract on lipid peroxidation and antioxidant activity in rabbits exposed to γ-radiation. Revue Med. Vet., 157: 415-419. www.revmedvet.com/2006/RMV157_415_419.pdf.

Fernández, V., L. Massa, L. Quiñones, K.A. Simon-Giavarotti, L. Giavarotti, V. D'Almeida, L.A. Azzalis, V.B. Junqueira and L.A. Videla, 2003. Effects of gamma-hexachlorocyclohexane and L-3, 3', 5-triiodothyronine on rat liver cytochrome P4502E1-dependent activity and content in relation to microsomal superoxide radical generation. Biol. Res., 36 (3-4): 359-65. PMID: 14631868.

Fidan, A.F. and Y. Dundar, 2008. The effects of Yucca schidigera and Quillaja saponaria on DNA damage, protein oxidation, lipid peroxidation and some biochemical parameters in streptozotocin-induced diabetic rats. Journal of Diabetes and its Complications. DOI: 10.1016/j.jdiacomp.2007.11.010.

Halliwell, B. and J.M.C. Gutteridge, 1998. Free Radicals in Biology and Medicine 3rd Edn. Oxford University Press, London. ISBN: 9780198500452.

Hayes, J.D. and L.I. McLellan, 1999. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radic Res., 31: 273-300. PMID: 10517533.

Jalili, S., M. Ilkhanipour, R. Heydari, A.A. Farshid and S. Salehi, 2007. The Effects of Vitamin E on Endosulfan-Induced Oxidative Stress in Rat Heart. Pak. J. Nut., 6 (4): 375-380.

Junqueira, V.B.C., K. Simizu, L.A. Videla and S.B.M. Barros, 1986. Dose-Dependent Study of the Effects of Acute Lindane Administration on Rat Liver Superoxide Anion Production, Antioxidant Enzyme Activities and Lipid Peroxidation. Toxicology, 41: 193-204. DOI: 10.1016/0300-483X(86)90199-X.

Junqueira, V.B.C., O.P. Koch, A.C.M. Arisi, A.P. Fuzara, L.A. Azzalis, S.B.M. Barros, A. Cravero, S. Farre and L.A. Videla, 1997. Regression of Morphological Alteration and Oxidative Stress-Related Parameters after Acute Lindane-Induced Hepatotoxicity in Rats. Toxicology, 117: 199-205. DOI: 10.1016/S0300-483X(96)03580-9.

Kappus, H., 1987. Oxidative stress in chemical toxicity. Arch. Toxicol., 60 (1-3): 144-149. PMID: 3304204.

Katz, D., D. Mazor, A. Dvilansky and N. Meyerstein, 1996. Effect of radiation on red cell membrane and intra cellular oxidative defense system. Free Radic Res., 24 (3): 199-204. PMID: 8728121.

Kerr, M.E., C.M. Bender and E.J. Monti, 1996. An introduction to oxygen free radicals. Heart and Lung: J. Acute Critical Care, 25 (3): 200-209. DOI: 10.1016/S0147-9563(96)80030-6.

Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic and V. Cosic, 2001. Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol., 54: 356-361. PMID: 11328833.

Krieger, T.R. and R.L. Caruso, 2001. Antioxidants prevent g-hexachlorocyclohexane-induced inhibition of rat myometrial gap junctions and contractions. Biol. Reprod., 64 (2): 537-47. PMID: 11159356.

Lightboy, J.H., L.M. Stevenson, F. Jackson, K. Donaldson and D.G. Jones, 2001. Comparative aspects of plasma antioxidant status in sheep and goats and the influence of experimental abomasal nematode infection. J. Comparative Pathol., 124: 192-199. DOI: 10.1053/jcpa.2000.0453.

Loch-Caruso, R., B.L. Upham, C. Harris and J.E.Trosko, 2005. Divergent Roles for Glutathione in Lindane-induced Acute and Delayed-onset Inhibition of Rat Myometrial Gap Junctions. Toxicol. Sci., 85 (1): 694-702. DOI: 10.1093/toxsci/kfi123.

Manahan, S.E., 2003. Toxicological Chemistry and Biochemistry. 3rd Edn. CRC Press LLC. USA Florida. ISBN: 1-56670-618-1.

Miranda, K.M., M.G. Espey and A.D. Wink, 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 5: 62-71. DOI: 10.1006/niox.2000.0319.

Misra, H.P. and I. Fridovich, 1972. The Role of Superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 10: 3170-5. PMID: 4623845.

Moslen, M.T., 1994. Reactive oxygen species in normal physiology, cell injury and phagocytosis. Adv. Exp. Med. Biol., 366: 17-27. PMID: 7771251.

Oberoi, S., R.S. Ahmed, S.G. Suke, S.N. Bhattacharya, A. Chakraborti and B.D. Banerjee, 2007. Comparative effect of topical application of lindane and permethrin on oxidative stress parameters in adult scabies patients. Clin. Biochem., 40 (16-17): 1321-1324. DOI: 10.1016/j.clinbiochem.2007.07.011.

Ohkawa, H., N. Ohishi and K.Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyt. Biochem., 95: 351-358. DOI: 10.1016/0003-2697(79)90738-3.

Repetto, R. and S.S. Baliga, 1996. Pesticides and the Immune System: The Public Health Risk. 1st Edn. World Resources Institute. Washington D.C. ISBN: 1-56973-087-3.

Rubbo, H., R. Radi, M. Trujillo, R. Telleri, B. Kalyanaraman, S. Barnes, M. Kirk and B.A. Freeman, 1994. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. J. Biol. Chem., 269: 26066-75. PMID: 7929318.

Safe, S.H., 1993. Lindane and Hexachlorocyclopentadiene. IN: Kroschwitz, J.I. and Howe-Grant (Eds.). The Kirk-Othmer Encyclopedia of Chemical Technology. 4th Edn. Pub. Wiley-Interscience, N.Y., 6: 135-139. ISBN: 0471419613.

Sen, S.K., 2000. Cellular thiols and redox-regulated signal transduction. Curr. Top. Cell. Regul., 36: 1-30. PMID: 10842745.

Sokal, R.R. and F.J. Rohlf, 1969. The Principles and Practice of Statistics in Biological Research. 1st Edn. W.H. Freeman and Company, pp: 469-84. San Francisco. ISBN: 0716706636

Suter, P., 1983. Three month toxicity study in rats with lindane. Unpublished report No. 005220 from Research Consulting Company AG, Itingen, Switzerland. Submitted to WHO by CIEL, Brussels, Belgium.

Vıdela, L.A., 2000. Energy metabolism, thyroid calorigenesis and oxidative stress: Functional and cytotoxic consequences. Redox. Rep., 5 (5): 265-75. PMID: 11145101.

Vıdela, L.A., S.B.M. Barros and V.B.C. Junqueıra, 1990. Lindane-induced liver oxidative stress. Free Radic. Biol. Med., 9 (2): 169-79. PMID: 1699849.

Videla, L.A., G. Smok, P. Troncoso, K.A. Simon, V.B.C. Junqueira and V. Fernandez, 1995. Influence of hyperthyroidism on lindane-induced hepatotoxicity in the rat. Biochem. Pharmacol., 50 (10): 1557-1565. PMID: 7503757.

Vidyasagar, J., N. Karunakar, M.S. Reddy, K. Rajnarayana, T. Surender and D.R. Krishna, 2004. Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning. Indian J. Pharmacol., 36: 76-79. http://www.ijp-online.com/text.asp?2004/36/2/76/6763.

Wang, P.G., B.C. Tingwei and N. Taniguchi, 2005. Nitric Oxide Donors. 1st Edn. WILEY-VCH Verlag GmbH and Co. KgaA, Weinheim, pp: 3-8. ISBN: 9783527603756. DOI: 10.1002/3527603751.

Žourek, M., P. Kyselová, J. Mudra, M. Krèma, Z. Jankovec, S. Lacigová, J. Víšek and Z. Rušavý, 2008. The Relationship between Glycemia, Insulin and Oxidative Stress in Hereditary Hypertriglyceridemic Rat. Physiol. Res., 57 (4): 531-538. PMID: 17705681.