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Araştırma Makalesi / Research Article

Ultrastructural and Biochemical Evaluation of the Effect of Endosulfan on Mice Liver

Endosülfan'ın Fare Karaciğeri Üzerine Etkisinin Ultrasütrüktürel ve Biyokimyasal Değerlendirilmesi

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ABSTRACT

Purpose: The aim of this study is to examine the effect of endosulfan on mice liver as ultrastructural together with biochemical data, and also by light microscopy to evaluate.

Materials and Methods: 12 male albino mice weighing between 26-30g were used. Mice were divided into two equal groups. For group receiving endosulfan, endosulfan (13 mg/kg/day, body weight) dissolved in nut oil was administered orally to mice via intragastric-during 10 days. The control group received only nut oil. At the end of the 10th day, all the mice were sacrificed with cervical dislocation. Tissue samples were taken for histological examination and biochemical analysis (SOD; superoxide dismutase, GSH; glutathione peroxidase, CAT; catalase, G6PD; glucose-6-phosphate dehidrogenase, MDA; malondialdehyde).

Results: Disruption of inner membrane of mitochondria, dilatation of endoplasmic reticulum, the presence of large lipid droplets and vacuolar structures were observed in some hepatocytes. Prominent vacuolization was seen in light microscopic observation. There was an increment in activities of G6PD, SOD, GSH and MDA. However, there was a decrease in CAT activity.

Conclusion: It may be considered that the adverse effect of endosulfan on mice liver may be due to direct toxic effect of endosulfan and induction of oxidative stress.

Key Words: ultrastructure, endosulfan, liver, toxicity

ÖZET

Giriş: Çalışmanın amacı, endosülfan'ın fare karaciğeri üzerine etkisini biyokimyasal veriler ile birlikte ultrasütrüktürel olarak araştırmak, ve aynı zamanda ışık mikroskobik değerlendirmek.

Materyal ve Metod: Çalışmada 26-30 gram ağırlığında 12 erkek albino fare kullanıldı. Fareler iki eşit gruba ayrıldı. Endosülfan grubuna, fındık yağında çözülmüş endosülfan (13 mg/kg/gün, vücut ağırlığ) 10 gün süresince intragastrik yol ile oral olarak uygulandı. Kontrol grubuna, yalnızca fındık yağı verildi. Bütün fareler onuncu günün sonunda servikal dislokasyon ile sakrifiye edildi. Histolojik ve biyokimyasal analizler (SOD; superoksit dismutaz, GSH; glutatyon peroksidaz, CAT; katalaz, G6PD; glikoz-6-fosfat dehidrogenaz, MDA; malondialdehid) için doku örnekleri alındı.

Bulgular: Bazı hepatositlerin mitokondrionlarının iç membranlarında bozulma, endoplazmik retikulum sisternalarında dilatasyon, lipid ve vakuoler yapıların varlığını gözlendi. Işık mikroskobik olarak belirgin vakuolizasyon görüldü. G6PD, SOD, GSH ve MDA aktivitelerinde artmaya rağmen CAT aktivitesinde azalma vardı.

Sonuç: Endosülfanın fare karaciğeri üzerine olumsuz etkisinin nedeni olarak, endosülfanın hepatositlere direkt toksik etkisi yanısıra oksidatif stres indüksüyonuna bağlı olabileceği düşünülebilir.

Anahtar Kelimeler: Ultrasütrüktür, endosülfan, karaciğer, toksisite

INTRODUCTION

Endosulfan, a polycyclic chlorinated hydrocarbon of cyclodiene group is an organochlorine insecticide, is important environmental contaminant. Nonetheless, endosulfan has been widely used in developing countries in destruction of agricultural pests in the agricultural industry[1,2]. Foods are the main source of exposure to endosulfan in general population. Endosulfan may enter to human or animals via either directly or environmental contamination. Endosulfan toxicity is due to uncontrolled use of endosulfan in agriculture or suicidal exposure. Endosulfan is readily absorbed from skin, lungs and gastrointestinal tract, so it is capable of high lethality or significant morbidity[3]. Endosulfan is stimulant of central nervous system, and its primary effect is neurotoxicity after long term inhalation or oral exposure[4,5]. The liver and kidney are the other organs significantly affected by endosulfan. Experimental studies have shown that endosulfan may lead to dilatation and degeneration of tubular epithelium in kidney, hydropic degeneration in liver, testicular damage and distortion of spermatogenesis[6,7]. Furthermore, it is said that reactive oxygen species may play an important role in toxicity of environmental chemicals, and may change antioxidant enzyme systems in cell[8]. As result of endosulfan exposure, the inhibition of microsomal enzymes inconnection with acute and subacute toxicity in liver is notified[9]. In the literature, the ultrastructural effects of endosulfan on liver and the correlation between the structural changes and antioxide system are uncommon. The aim of this study is to evaluate possible effects of endosulfan on mice liver as electron and light microscopic together with biochemical data.

MATERIAL AND METHODS

Twelve adult male albino mice weighing between 26-30 g were used in this study. All mice were obtained from the Medical Sciences Experimental Research Center of the University of Çukurova. The experiment was performed in accordance with guide for the care and use of laboratory animals. Throughout the experiment, all mice were fed a standart laboratory diet at normal room temperature (24±1ºC) and humidity (60-65%). Mice were housed in steel cages with a 12 h light-dark cycle (lights on at 6.00 a.m.). Mice were divided equally into two groups. Group 1: control group (n=6). Group 2: experimental group (n=6). Mice comprising the experimental group received endosulfan dissolved in nut oil at a dosage of 13 mg/kg/day body weight. Endosulfan was administered orally to mice via intragastric during 10 days. The control group received only nut oil. At the end of 10th day, all the mice were sacrificed with cervical dislocation. Liver tissue was quickly removed for microscopic examinations and biochemical analyses. Body weight of all animals was determined at the end of the experiment (data not shown).

Electron Microscopic Analysis

Liver tissue samples were immediately placed in 5% glutaraldehyde buffered at pH 7.4 with Milloning phosphate buffer for 4 hours and subsequently fixed in 1% osmium tetraoxide for two hours. The samples were dehydrated in graded ethanol and embedded in araldite. Thin sections were stained with lead citrate and uranyl acetate, and examined with a Zeiss EM 10B and JEOL JEM 1400 transmission electron microscope.

Light Microscopic Analysis

Semithin sections in 1 µm-thick was obtained from liver tissue samples embedded in araldite , mounted on slides. Semithin sections was stained in 1% toludine blue, and then were examined by light microscopy.

Biochemical Analysis

The liver tissue samples were stored at -20º C until the biochemical studies. Liver samples

were homogenised with three volumes of ice-cold 1.15% KCI. The activities of antioxidant enzymes (G6PD, SOD, CAT, GSH) and MDA were measured in the supernatant obtained from centrifugation at 4000 rpm. SOD (superoxide dismutase) activity was measured in the tissue samples according to the method described by Fridovich [10]. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with p-iodonitrotetrazlium violet (INT) to form a red formazan dye, which was measured at 505 nm. SOD activity was expressed as U/g tissue. CAT (catalase) activity was determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler[11]. CAT activity was expressed as U/g tissue. Activity of G6PDH (glucose-6-phosphate dehydrogenase) was determined at 37° C in the tissue samples according to method of Beutler[11] . It was determined by the increase of absorbance at 340 nm of NADPH (nicotinamide adenine dinucleotide phosphate). G6PDH activity was expressed as U/g tissue. GSH (glutathione) level was measured in the tissue samples according to Beutler[11]. The reaction mixture contains filtrate, phosphate buffer and DTNB (5,5'-dithiobis 2-nitrobenzoic acid) in a final volume of 10 ml. The absorbances are read in a spectrophotometer immediately at 412 nm. GSH level was expressed as µmol/g tissue. MDA (malondialdehyde) level in the tissue samples was measured with the TBA (thiobarbituric acid) test by the method of Ohkawa[12] . After centrifugation, the absorbance of the organic layer was measured at 532 nm. MDA level was expressed as nmol/g tissue.

Statistical Analysis

The SPSS programme (Version 9.05) was used for Mann-Whitney U test. Results were expressed as the mean (X) ± standard deviation (SD). The difference was considered significant when the probability was less than 0.05, ($p<0.05$).

RESULTS

Ultrastructural Findings

Electron microscopic observation of the liver tissue in the control group appeared normal ultrastructure. In micrographs, nucleous and cytoplasmic organelles in the liver cells were normal. Additionally, glycogen deposits in the hepatocyte cytoplasm are seen as regular electron dense particles (Fig. 1). In endosulfan-treated group, although some of the liver cells exhibit normal structure, most of cells showed slight and moderate ultrastructural alterations. Dilatation of endoplasmic reticulum cisternae was seen (Fig.2). There was prominent mitochondrial degeneration in some of hepatocytes. Swelling of mitochondria with dissolution of the cristae was seen. Furthermore, there was large lipid droplets. Moreover, the presence of vacuoles in some lipid droplets were striking findings in some hepatocytes (Fig. 3).

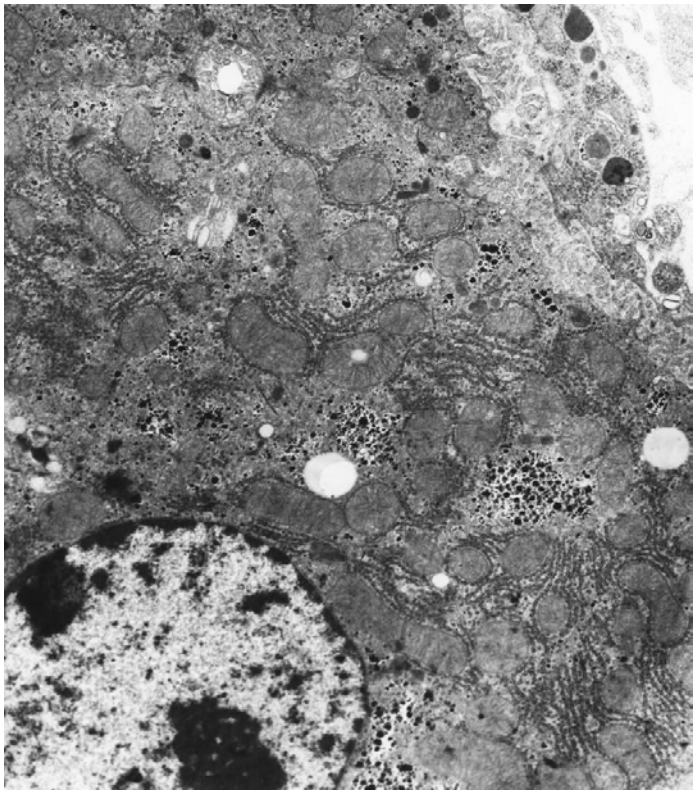

Figure 1. Control group. The liver cell shows normal ultrastructure. The nucleus (N), mitochondria (M), endoplasmic reticulum cisternae (ER), glycogen particles (G), lipid droplets (L), bile canaliculi (BC) and Disse space (DS) are indicated. (original magnification, x 12.400).

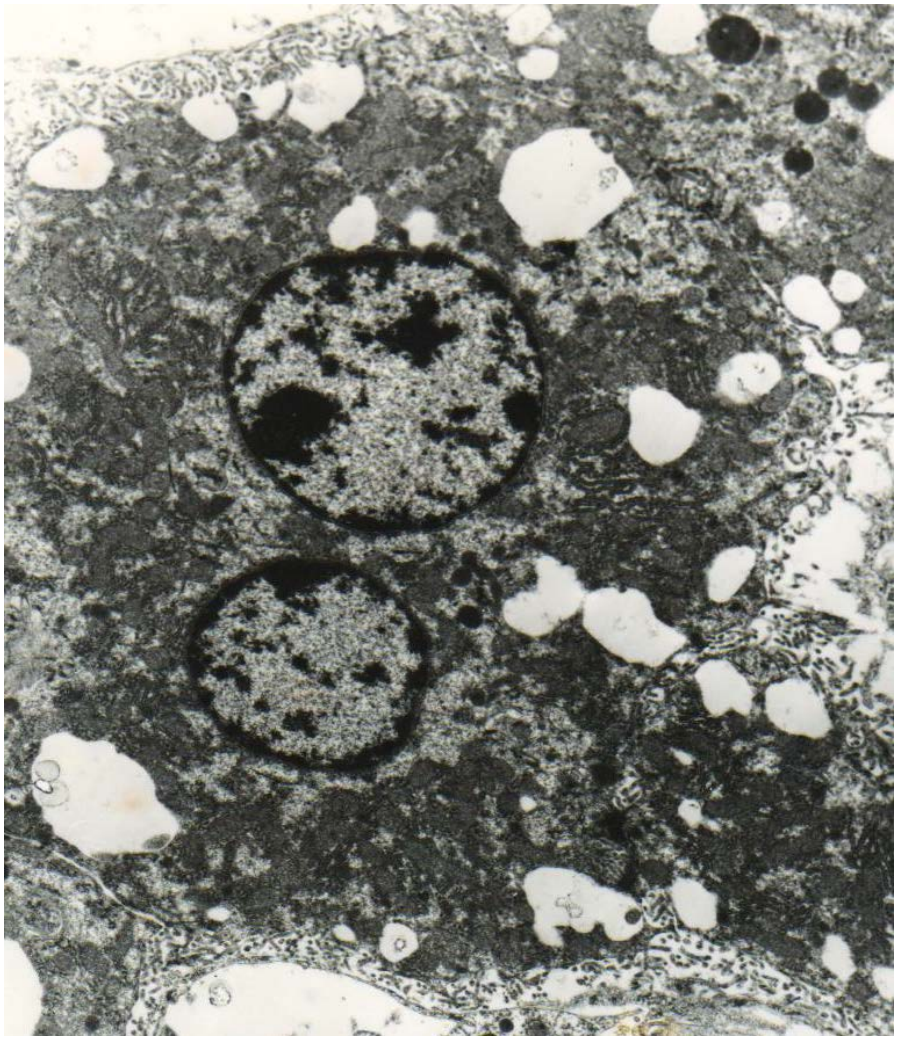

Figure 2. Endosulfan-treated group. The liver cells reveal enlargement of endoplasmic reticulum cisternae (ER). The nuclei (N), lysosomes (LS), Disse spaces (DS) and sinusoidal capillary (SC) are indicated. (original magnification, x 12.400).

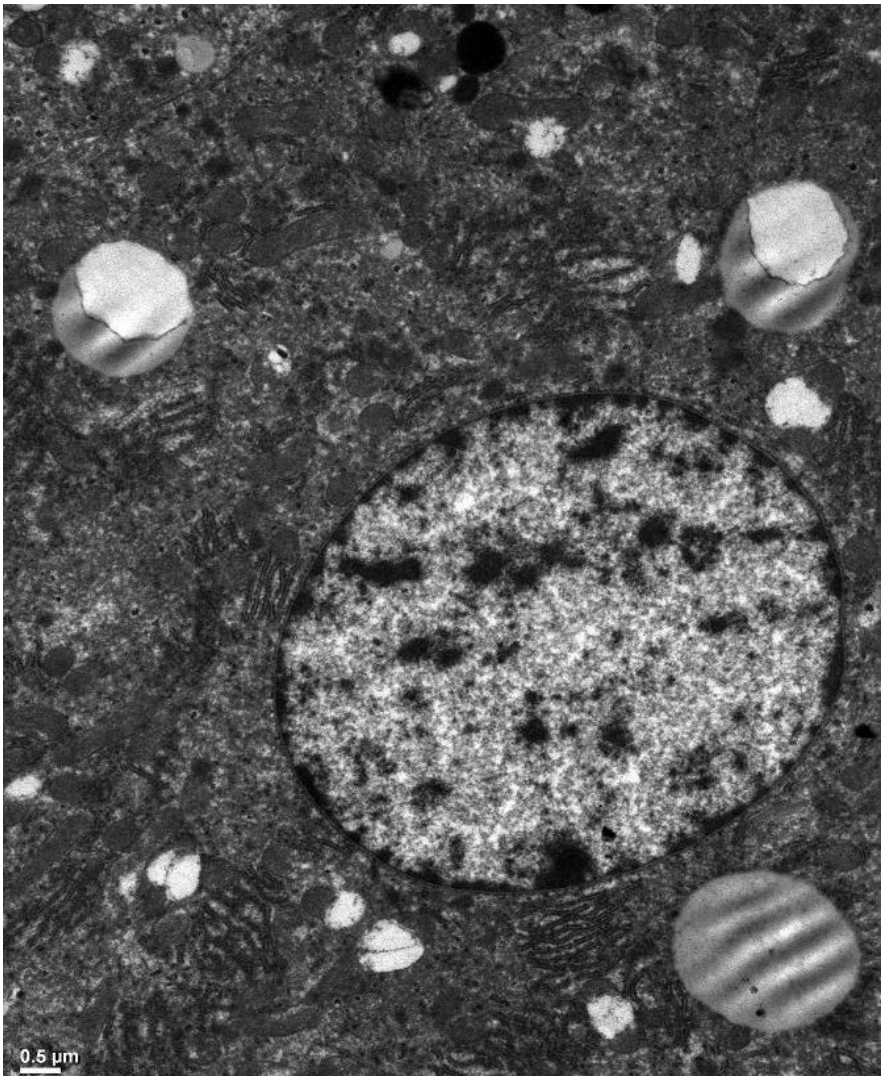

Figure 3. Endosulfan-treated group. The liver cell exhibits swelling of mitochondria (M) and disruption of inner membranes of mitochondria . The nucleus (N), large lipid droplets (L). The presence of vacuoles (asterisks) in some of lipid droplets are indicated.

Light Microscopic Findings

As a result of light microscopic observation of semithin sections, the control group showed normal polygonal hepatocytes with round-shaped nuclei and hepatic sinusoids located in between the hepatic cords (Fig.4). In endosulfan-treated group, prominent vacuolization in hepatocytes was striking. Besides of the liver cells in normal structure, the presence of enlarged nuclei and an increase in size of some of the hepatocytes was observed. Sinusoids were slightly dilated (Fig.5).

Biochemical Findings

The values of activities of G6PD, CAT, SOD, GSH, and MDA are shown in Table 1. Values of biochemical analysis of the experimental group were statistically significant when compared with the control group values. There was increase in values of activities of G6PD and SOD enzyme. However, there was prominent a decrease in value

of CAT enzyme. Furthermore, an increase in the levels of GSH and MDA was seen in experimental group compared to control.

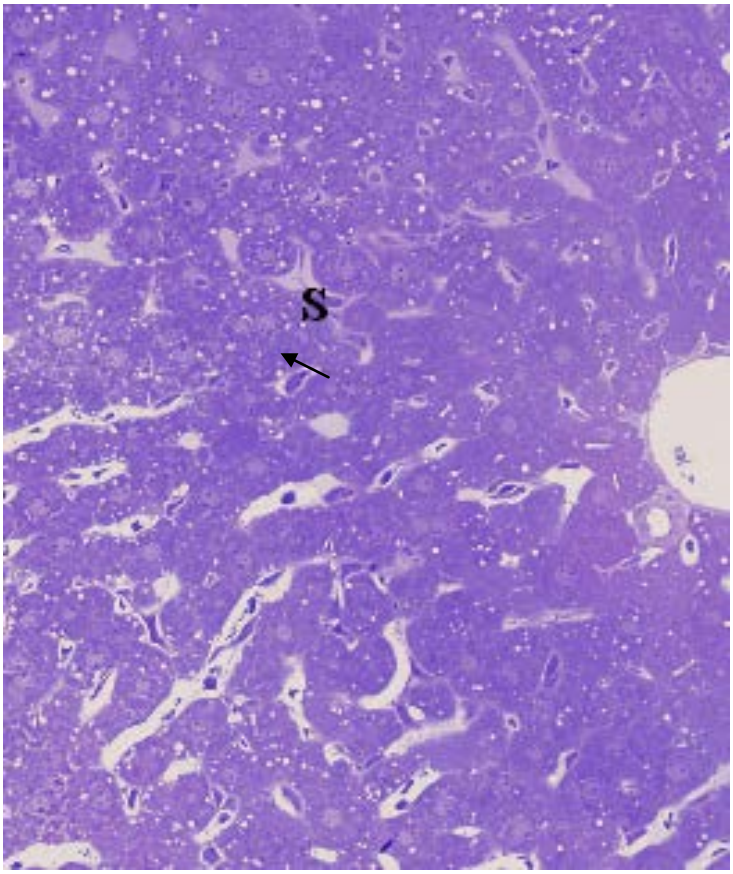

Figure 4. Control group. Liver cells exhibit normal light microscopic structure. Sinusoides (S), vesicular nuclei (arrows), central ven (CV) are indicated. (Toluidine blue, x 20).

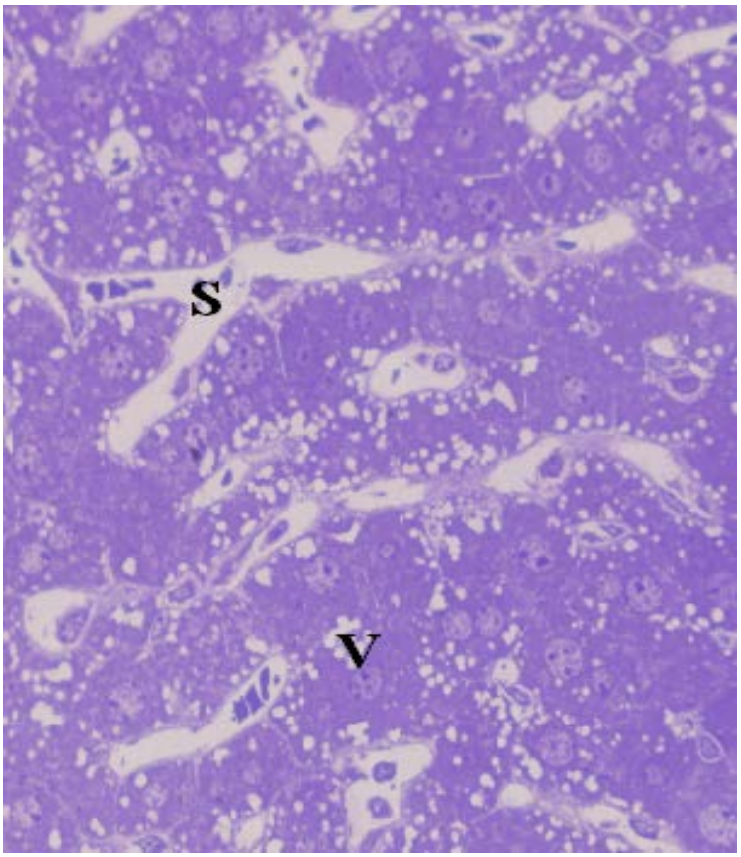

Figure 5. Endosulfan-treated group. Dilated sinusoids (S), prominent vacuolar structures in various size in most of the liver cells (V), the presence of enlarged nuclei and an increase in size of some hepatocytes (arrows) are indicated (Toluidine blue, x 40).

Table1. The values of biochemical studies as statistical.

	Control Group (X±SD)	Experimental Group (X±SD)
G6PD (U/g tissue)	2,21±0,78	4,49±2,75
CAT (U/g tissue)	3805,16±748,34	2079,16±690,75
SOD (U/g tissue)	814±365,42	1372,00±359,44
GSH (umol/g tissue)	0,93±0,22	1,86±0,68
MDA (nmol/g tissue)	21,83±5,56	40,00±11,91

All data are expressed as mean (X) ± standard deviation (SD) from six mice in each group. Increase in G6PD, SOD, GSH, MDA, and decrease in CAT compared with control group was significant ($p < 0,05$)

DISCUSSION

Nonactive foreign chemical substances point of view biological effect organs as transforming into active toxic metabolites in specific organs. Liver is the main organ metabolizing chemicals. Reactive metabolites such as endosulfan sulfate and endosulfan diol may be formed during metabolism of the endosulfan in liver, and these metabolites may be toxic for the cells[13]. In experimental studies have been reported that

metabolites of endosulfan may impair configuration of the cell membrane[14,15]. Free radicals are broken products of the chemical generated by cytochrome P450-mediated metabolism in the liver. It is said that the principle metabolites of endosulfan may cause lipid peroxidation damage in cell[16,17].

In our micrographs, swelling and disruption of inner membranes of mitochondria was seen in experimental group. It may be thought that metabolites of endosulfan may have a direct effect on the cell membrane. Thus, endosulfan may disrupt homeostasis of cell by effecting energy production of cell. Yamano et al.[18] reported that trichlamide hepatotoxicity may be associated with depleted cellular ATP content. Furthermore, membranes of endoplasmic reticulum and mitochondria are rich from unsaturated fatty acid. It may be said that lipophilic metabolites of endosulfan may impair the structure of mitochondrial membrane, and it may be cause mitochondrial disfunction. Suziki et al.[19] suggested that metabolites of fungicides produced by microsomal cytochrome system may cause peroxidation of membrane phospholipids, causing cytotoxicity.

In our study, enlargement of the endoplasmic reticulum cisternae was also seen. Most of enzymes containing cytochrome P450 are located in endoplasmic reticulum cisternae[20]. Endosulfan has been shown to cause induction of hepatic cytochrome P450 system in experimental animals[21]. According to our findings, morphological changes in endoplasmic reticulum can be seen as dilatation. These alterations may be related with reactive metabolites which can occur during cytochrome P450-mediated metabolism. Sharma et al.[22] reported that elevated levels of cytochrome P450 may be a sign of radicals production in high rates, and free radicals increasing in the cell may cause to lipid peroxidation. Furthermore, in our micrographs, large lipid droplets containing vacuoles in some of hepatocytes was seen. These alterations can be related to accumulation of triglycerides as resulting of degeneration of liver metabolism. Pacheco et al.[23] reported that some environmental contaminants may be effective as serious on lipid metabolism. Exposure to pesticide can lead to oxidative stress due to unregulated generation of reactive oxygen species (ROS). Under normal conditions, antioxidant systems of the cell minimize damage caused by ROS. When ROS generation increases in the cell, the result is the oxidative stress[24,25]. According to our biochemical findings, endosulfan metabolites may effect the membrane enzymes, and may lead to the formation of superoxide radicals in the cell. Superoxide radicals in the cell are broken by SOD. Thus, activity of SOD increasing in the cell may be indicator of a defence against superoxide radicals. Radosavljevic et al.[26] reported that SOD activity increased significantly with the liver toxicity of lindane, and they said that this situation may related with oxidative stress.

In our study, endosulfan caused possibly negative effect by decreasing CAT activity in the cell. Catalase (CAT), the main antioxidative enzyme, converts hydrogen peroxide (H_2O_2) into O_2 and H_2O. As a result, the destruction of hydrogen peroxide is provided by CAT[27,28] . Thus, the cytotoxic effect of H_2O_2 is important for the cell. Therefore, a decrease in activity of CAT and increase in activity of MDA may reflect the presence of decreasing free radicals in the liver cells. On the other hand, an increase in GSH and G6PD is possibly showed defence towards forming free radical in the cell. But, the inability to antioxidant defence system may contrib.ute in occuring of the cell injury. Thus, reactive oxygen species may play an important role in liver injury.

Finally, in the evaluation together with ultrastructural and biochemical alterations on the liver, direct toxic effect of metabolites of endosulfan and possible increasing in oxidative stress during metabolism of endosulfan can be considered to play a role in cell injury.

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