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Dose-Dependent Effects of Endosulfan and Malathion on Adult Wistar Albino Rat Ovaries

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Abstract: In this study, histological effects and malondialdehyde (MDA) levels were investigated by endosulfan and malathion in adult female rat ovaries. An increase of MDA level in rat ovarium tissues due to endosulfan and malathion may be an indicator of the free radicals occurred during the metabolism and their lipid peroxidative inducing damage. In this study in accordance with the biochemical findings, the study demonstrated that there are pronounced structural defects in histological examinations of ovarian tissues in rats which were administered endosulfan and malathion. It has been observed that the size of ovarian tissues of rats which were administered endosulfan and malathion in different doses was decreased in various levels. There was a significant decrease healthy follicles and a significant increase atretic follicles in low dose of endosulfan and malathion (11 mg kg^{-1}) treated rats. The histologic observations of the ovary revealed the presence of less number of healthy follicles and more number of atretic follicles and corpus luteums in high dose of endosulfan and malathion (33 mg kg^{-1}) treated rats. Finally, a peroxidative damage occurs inavitably due to endosulfan and malathion for ovarium tissues. The biochemical results (MDA levels) also showed such a damage, similar with the histological results.

Key words: Histopathological effects, malondialdehyde, pesticide

INTRODUCTION

Pesticide differ from any other chemical substances because they are deliberately spread into the environment. As a consequence, a great part of the human population may be exposed either in the general environment or in the working settings. While the environmental exposure, involving general population is mainly due to the ingestion of the contaminated foods, water and respiratory route (Baligar and Kaliwal, 2001). In recent years, there have been growing concerns about the toxicity of a number of chemicals, including pesticides, on the female reproductive system. Such two pesticides are endosulfan and malathion, which are widely used in Turkey to maintain crop and food production, to fight against infesting pests and to safeguard humans from vector borne diseases and related epidemics (WHO, 2006). Endosulfan is an organochlorine insecticide. There are some major health concerns regarding this type of insecticides due to their prolonged persistence in the body. Endosulfan, however, has low residual persistence and hence had been preferred over other insecticides in this group. The dose and duration of exposure to endosulfan are important determinants in its cellular and biochemical toxicity (Hiremath and Kaliwall, 2002). Malathion is an organophosphate compound and one of the most widely used organophosphate insecticides throughout the world. It is used to control pests affecting agricultural crops, ornamentals, greenhouses, livestock, stored grain, forests, buildings, households and gardens. Contributing to its popularity is its relatively low acute mammalian toxicity (Kaur and Dhanju, 2005). In this study, comparison of low and high doses of endosulfan and malathion were administrated intraperitoneally to the female rats for 15 days to investigate the biochemical and histological effects on ovaries of rats.

MATERIALS AND METHODS

Animals: Adult (n = 50) female Wistar albino rats (150-200 g) were obtained from Marmara University, Center for Animal Breeding, Istanbul, Turkey and study was conducted from 2006 to 2007. The rats were divided at random into four groups of 10 animals each. They were maintained under a well regulated light and dark (12:12 h) schedule at 24±3°C and were allowed free access to laboratory chow and tap water.

Animal treatments: Sixty four rats, weighing 200-280 g at 12 weeks of age, were divided into five groups of ten. The experimental groups in this study were as follows: Low dose of endosulfan (E1), high dose of endosulfan (E2), low dose of malathion (M1), high dose of malathion (M2) and control group (C). Fifteen doses (15 days) were

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administered via., intraperitoneally way to 50 rats in each treatment group at the following levels: 11 mg kg^{-1} (10% of LD_{50}) endosulfan and malathion for E1 and M1 and 33 mg kg^{-1} (30% of LD_{50}) endosulfan and malathion for E2 and M2, respectively. An equal amount of physiologic saline was also given to control (C) rats. The rats in all treatment groups were killed by cervical dislocation after 15 days for cronical effects. The tissue samples of ovaries were taken by using appropriate techniques for histological and biochemical examination. The level of MDA were determined in ovarium tissue samples of rats.

Tissue preparation: The ovaries were fixed in Bouin's solution for 8 h. Fixed tissues were dehydrated and embedded in paraffin wax and sectioned transversely at 5 μm thickness and stained with Hematoxylin eosin and Masson trichomic stain. The periodic acid Schiff reaction (PAS) was applied. Samples were evaluated by examining under light microscope. These protocols were approved by the Animal Ethical Committee University of Marmara, Faculty of Medicine.

Malondialdehyde (MDA) assays: After decapitation, the ovarium tissue samples were immediately taken using appropriate techniques for biochemical examinations and stored in deep-freeze at -20ºC. Afterwards, MDA levels, an end product of lipid peroxidation were measured in these tissue samples. Tissue samples were homogenized ice-cold 150 mM KCl in a tissue homogenizer for determination of malondialdehyde levels. Results were expressed as nmol g^{-1} MDA tissue (Buege and Aust, 1987; Draper and Hadley, 1990).

RESULTS AND DISCUSSION

It has been observed that the size of ovarian tissues of rats which were administered endosulfan and malathion in different doses was decreased in various levels. When compared to the controls, it was showed that ovaries especially in groups which were administered high doses of endosulfan and malathion (E2 and M2), thoroughly were smaller than the others. In histological examinations of ovaries in rats, general morphology and cell morphology of ovaries were taken into account. The histologic observations of control group showed developing follicles (primordial, primary and secondary follicles), corpus luteum and graaf follicles were observed in the cortex of ovarium (Fig. 1A). Primordial follicles are composed of an oocyte surrounded by a small number of squamous granulosa cells. Structural morphology of healthy oocytes in developing follicles appears similar

Table 1: Mean levels of malondialdehyde (MDA: nmol g^{-1}) levels of adult rat ovaries

Experimental groups	E1*	E2*	M1*	M2*	C*
	(nmol g^{-1})				
Ovary tissue	34.25±1.0	45.21±2.1	35.14±2.0	47.20±1.8	21.11±1.8

*E1: Low dose endosulfan, *E2: High dose endosulfan, *M1: Low dose malathion, *M2: High dose malathion, *C: Control group

regardless of the stage of development. This is tightly apposed to both the oocyte and the surrounding granulosa cell layer (Fig. 1F). The histopathological examination revealed that the most remarkable change in the ovaries was widespread ovarian follicle atresia accompanied by a decreased number of normal antral and growing follicles. According to Osman (1985), a follicle was considered to be undergoing atresia or to regressing whenever two or more pyknotic granulosa cells would be found in a single section or whether the oocyte showed signs of degeneration, or thinning of cumulus oophorus as purposed (Osman, 1985). Most of the follicles were atretic and viable oocytes were hardly found in the remaining follicles. Thin layer of granulosa cells were sometimes maintained in the remaining follicles. However, the number of corpus luteum was considerably fewer than in the control. In ovaries of rats which were administered endosulfan and malathion in low doses (E1 and M1), graaf follicles and atretic follicles were increased (Fig. 1B, C; 2A, C). On the other hand, in the high dose groups (E2 and M2) corpus luteum and atretic follicles were observed sorely (Fig. 1D, E; 2B, D). Treatment with 33 mg/kg/day malathion and endosulfan caused a significant decrease in the number of healthy follicles with concomitant significant increase in the number of atretic follicles and accordingly the corpus luteum area was enlarged due to dose increments for both pesticides.

To our knowledge, this is the first study to show that endosulfan and malathion affects numbers of primordial, primary and prenatral follicle, but it increases atretic follicle and corpus luteum. Various researchers have reported in some researches, that in the results of some pesticide administrations to females degenerations had been observed in ovaries related to dose (Alvarez *et al*., 2000; Baligar and Kaliwal, 2001; Chapin *et al*., 1997; Gray *et al*., 1989; Ishmael and Lithfield, 1988; Kaur and Dhanju, 2005). In this study in accordance with the biochemical findings, we demonstrated that there are pronounced structural defects in histological examinations of ovarian tissues in rats which were administered endosulfan and malathion. In groups of E1 and M1 (11 mg/kg/day), it was observed that external morphology of ovaries were almost normal. On the other hand, in high dose endosulfan and malathion groups (E2 and M2 33 mg/kg/day), it was seen that ovaries were

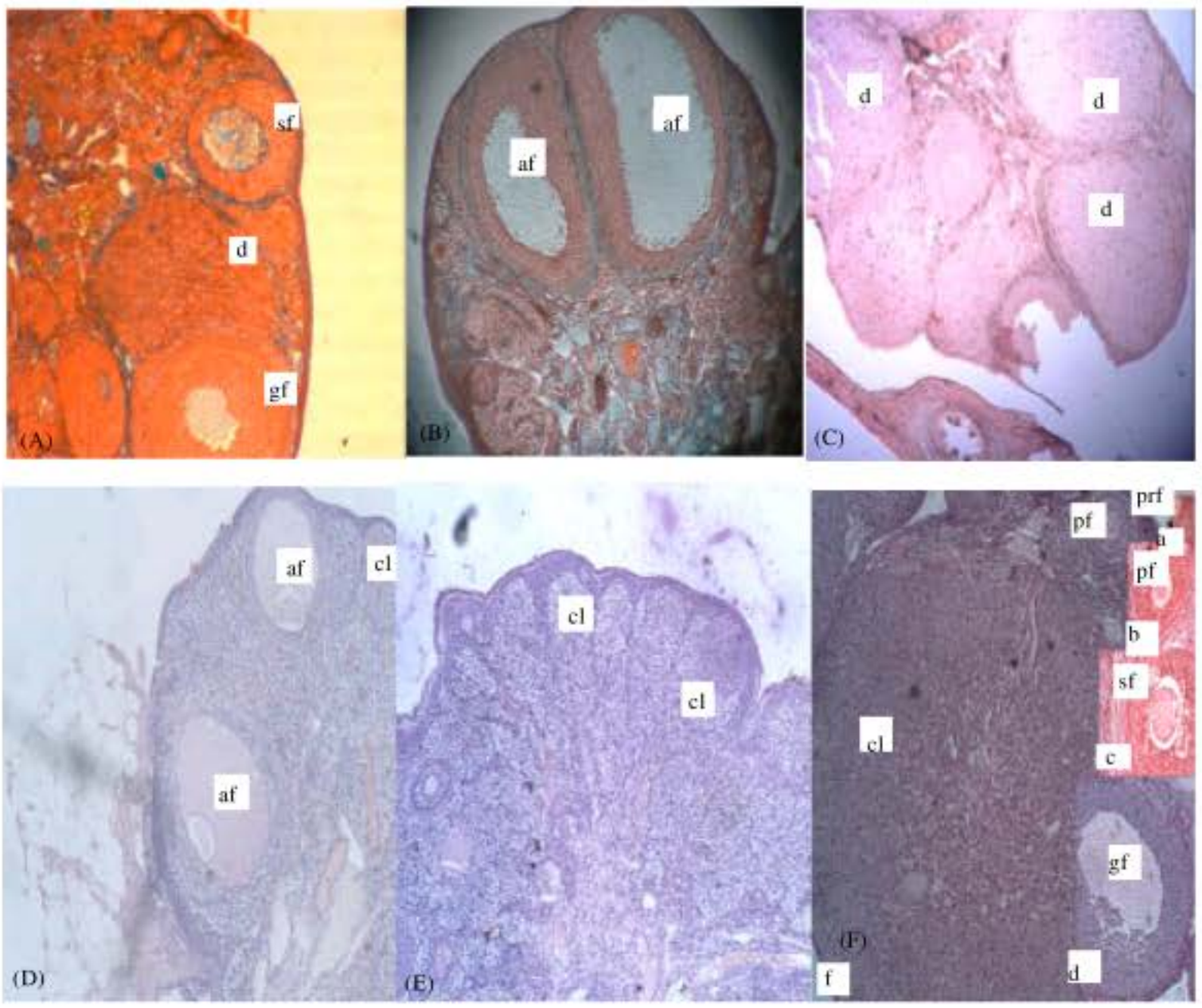

Fig. 1: (A) Photomicrograph of a tissue section of ovary from control group (C). Secondary follicle (sf), corpus luteum (cl), graaf follicle (gf). X10 Masson tricrom stain; (B) Photomicrograph of a tissue section of ovary from E1 treated group. Corpus luteum area was enlarged. Atretic follicle (af) was seemed. X10 Masson tricrom stain; (C) Photomicrograph of a tissue section of ovary from E2 treated group. Corpus luteum (cl) area was enlarged. X10 PAS stain; (D) Photomicrograph of a tissue section of ovary from M1 treated group. Atretic follicle's number (af) was increased. X10 HE stain; (E) Photomicrograph of a tissue section of ovary from M2 treated group. Corpus luteum (cl) and atretic follicle's number (af) was increased. X10 HE stain and (F) Photomicrograph of a tissue section of follicles of ovary from control group (C). Primordial follicles (prf), primary follicles (pf), secondary follicle (sf), corpus luteum (cl), graaf follicle (gf). a, f-X10 Masson tricrom stain, d-X40 H and E, b, c-X10-PAS

considerably smaller and the color was light when compared to controls. On the basis of histological examinations, it was showed that the number of corpus luteum and atretic follicles were increased. It was reported that various pesticide types (mancozeb, carbofuran, chlorodecon) in increasing doses lead to decreases in healthy follicle numbers and accordingly increases in atretic follicle numbers (Rockett *et al*., 2006; Kaur and Dhanju, 2005; Swartz and Mall, 1989). Methoxychlor is a known reproductive toxicant with the capability of accelerating female pubertal development and delaying male pubertal development when given at high doses (Chapin *et al*., 1997; Gray *et al*., 1989).

Plowchalk *et al*. (1993) have reported that the quantitative assessment of follicle number is an indicator of the normal function as well as toxic responses in the ovary. Follicles are the principle functional units of the mammalian ovary. The most important controllers of follicular development are follicle stimulating hormone and luteinising hormone produced from the pituitary and the ovarian steroid estradiol produced by granulosa cells (Plowchalk *et al*., 1993). The present study revealed that healthy follicles were significant decreased with concomitant significant increase atretic follicles and corpus luteum areas in higher doses. Similar finding have been reported on the reduction of different types of healthy follicular stages with concomitant increase in the atresia in rats and mice treated with different pesticides. It has been reported that the chlorinated pesticides induces follicular toxicity in reducing the pool of healthy, large

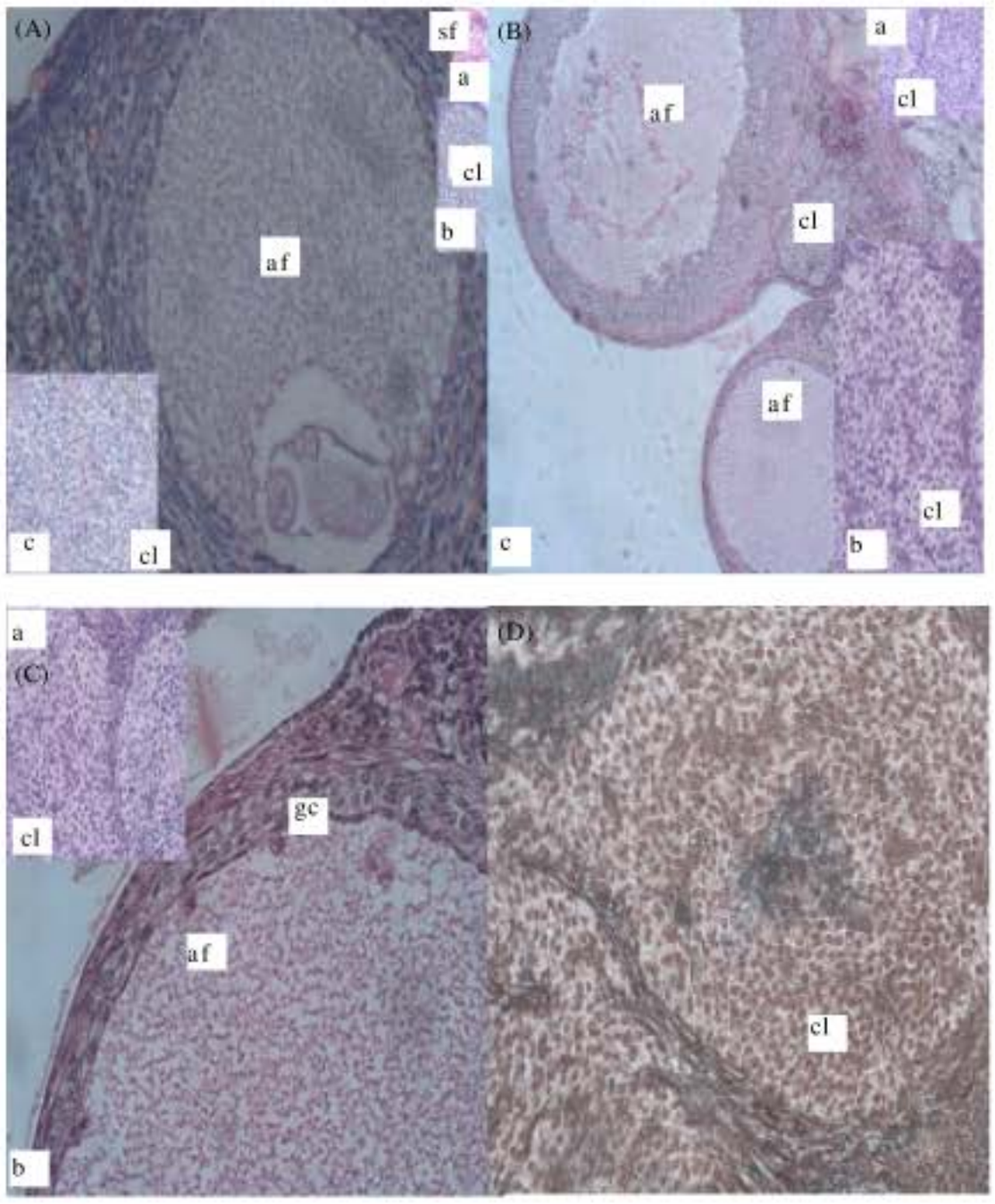

Fig. 2: (A) Photomicrograph of a tissue section of follicles of ovary from E1 treated group. Corpus luteum area was enlarged. Atretic follicle (af) was seemed. Seconder follicle (sf), corpus luteum (cl). a, b, c-X10 H and E, d-X40 H and E; (B) Photomicrograph of a tissue section of follicles of ovary from E2 treated group. Corpus luteum (cl) area, atretic follicle (af) was seemed. a, b, X10c-X40 H and E; (C) Photomicrograph of a tissue section of follicles of ovary from M1 treated group. Corpus luteum (cl) area, atretic follicle (af). A X10,b-X40 H and E and (D) Photomicrograph of a tissue section of corpus luteum from M2 treated group. Corpus luteum (cl) area. X40 Masson tricrom stain

sized follicles with increase in the atretic follicles (Jadaramkunti and Kaliwal, 1999; Martinez and Swartz, 1991; Swartz and Mall, 1989). Swartz and Mall (1989) examined the effect of chlordecone on follicle growth in their study and they reported that atretic and ovulatory follicle numbers are increased. In the result of histological evaluation in rats which were administered mancozeb in high doses, it has been noted that healthy follicle numbers and developing follicle numbers are decreased however atretic follicle numbers are increased. These data from different types of pesticides are in accordance with present study (Swartz and Mall, 1989). Alvarez *et al.* (2000) examined the toxic effects of the pesticide, hexachlorobenzen (HCB) on reproductive system of female rats. According to Alvarez *et al.* (2000), HCB caused to increase in estrogen levels in female rats. In the result of histologic examinations, degenerations on ovarium follicles and increase in atretic follicles were observed. These results are consistent with the histological findings (Alvarez *et al.*, 2000). Hiremath and Kaliwall (2002) examined the effects of endosulfan in albino female rats. According to Hiremath and Kaliwall (2002) endosulfan caused to decrease on ovarium functions. Furthermore, increase in atretic follicles were observed. Also, endosulfan showed similar effects in this study (Hiremath and Kaliwall, 2002). In a study of Baligar and Kaliwal (2001) numerous corpus luteums and atretic follicles were observed in ovarium. They noted that estrogen cycles were affected and cycle numbers were decreased accordingly. Based on the data obtained from a study investigating the healing effect of E and C vitamins and ovarium toxicity of methidathion in rats,

tissue damages on ovaries were reported. According to investigator, although there are positive effects of E and C vitamins, tissue damages on ovaries were observed and also MDA levels are increased related to methidathion dose (Baligar and Kaliwal, 2001). The dose related tissue damage and increase in malondialdehyde levels were supports present study. In another study examining the effects of fungicides on reproductive growth in female rats, the pesticides named as propiconazole, myclobutanil and triadimefon were used (Güney *et al*., 2007). It was reported that there are toxic effects of all these fungicides on ovaries of female rats (Plowchalk *et al*., 1993). According to the study investigating the biochemical effects of monocrotophos, dimethoate and methylparathion on ovaries of albino female rats, all pesticides caused degenerative changes on ovaries and the increase of total protein, lipid and cholesterol. All these changes were attributed to the toxic effect of pesticides in cellular and molecular levels on ovarium functions in rats (Kaur and Dhanju, 2005). In conclusion, in this study, tissue damages in ovarium tissues of rats because of endosulfan and malathion were examined by histologic and biochemical methods. From biochemical aspect, MDA levels in ovarium tissues of female rats are increased due to endosulfan and malathion and this finding was accepted as an indicator of free radicals and their lipid peroxide damages occurred during these insecticides metabolism. In accordance with the biochemical findings, we established major structural defects in histologic examinations of ovarium tissues of rats administered endosulfan and malathion. It has been observed that the size of ovarian tissues of rats which were administered endosulfan and malathion in different doses was decreased in various levels. In comparison to the controls, it was showed that ovaries especially in groups which were administered high dose of endosulfan and malathion thoroughly were smaller. In histological examinations of ovaries in rats, general morphology and cell morphology of ovaries were taken into account. In control group, developing primordial, primary and secondary follicles, corpus luteum and graaf follicles were observed in the cortex of ovarium. All biochemical and histologic findings from this study appears to be similar and compatible with the literature. As a result, tissue damage occurs in ovaries due to endosulfan and malathion.

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