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Histopathological Effects of Malathion and Endosulfan on Blood Cells of Wistar Albino Rats (*Rattus norvegicus*)

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Abstract

The aim of this study is an investigation of histopathological effects of malathion and endosulfan such as widely used insecticides in Turkey, on blood cells of wistar albino rats. In this study, we have used 20 wistar albino rats. These studies showed that erythrocyte cells have structural defects, degenerative cell size and surface shapes and also discomformity on membrane surfaces in rats administered endosulfan and malathion. It was also observed that these histopathological defects are related to dose increments. It has been demonstrated that the disconformity of leukocyte membranes is more pronounced when compared to erytrocyte membranes. It was also observed that the blood cells (leukocytes and erythrocytes) of rats in control group have a normal histological appearance. In conclusion, we have observed deformations in nucleus and cytoplasms of blood cells caused by different doses of malathion and endosulfan.

Key words: *Endosulfan, Malathion, Blood cells, Wistar albino rats*

INTRODUCTION

Many insecticides were completely banned in Turkey beginning from the 1990's. However, in spite of these bans, total pesticide usage in Turkey in 1995 was 37.000 tons and this usage has shown a steady increase year by year. Endosulfan and malathion are widely used in Turkey as pesticides to maintain crop and food production, fight against pest infestations, and to safeguard humans from vector-borne diseases and related epidemics. Endosulfan is an organochlorine insecticide currently used as an insecticide worldwide, and its residues are posing a serious environmental threat. This group of insecticides attracts major health concerns due to prolonged persistence in the body. Endosulfan, however, has low residual persistence and hence has been preferred over other insecticides in this group [1].

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 [2,3]. It has been reported that in vitro exposure of human bone marrow cells to metabolites of pesticides with organic phosphorus, erythroid and myeloid cells are suppressed; however, if they are administered in high doses, bone marrow cells return to normal. Blood accurately and completely reflects all physical and chemical changes in organisms [4]. Therefore blood analysis has an extended application area in fish and mammalians with metabolic disorders and septicemia. These analyses have importance because physiological changes due to the immunostimulants can be easily evaluated. In this study, we investigated the effects of endosulfan and malathion on blood cells of rats.

MATERIALS AND METHODS

Animals: Adult (n=20) Wistar albino rats (150-200 g) were obtained from Marmara University, Center for Animal Breeding, Haydarpasa, Istanbul, Turkey. The rats were divided at random into four groups of 3 animals each. They were maintained under a well regulated light and dark (12h:12h) schedule at 24ºC ± 3ºC and were allowed free access to laboratory food and tap water. The experimental groups in the present study were as follows: low dose of endosulfan (Group I), high dose of endosulfan (Group II), low dose of malathion (Group III), high dose of malathion (Group IV) and control group (Group V). The test group was given intraperitonial endosulfan dissolved in physiological saline solution at a dose of 1.0 mg/kg body weight for 15 days. The control animals received a similar volume of the vehicle. Twenty-four hours after the last treatment, the rats were weighed and put down using ether anesthesia. The protocols were approved by the Animal Ethical Committee of Marmara University, Faculty of Medicine.

Histopathology of blood cells : In this study, blood smear preparation technique was used and microscopic examination was carried out. After hematologic analysis of samples had been performed, one drop of blood sample was placed and then spread on the lam. After the preparations are dried for an hour, they are kept in maygrünwalt fixation substance for 15 minutes and then washed with distilled water. The preparations are stained with giemsa for 20 minutes, which is diluted by distilled water in 1/10 ratio. Then the stained preparations are examined by light microscope to determine the morphological changes in blood cells [5].

RESULTS

In this study, endosulfan and malathion were administered intraperitoneally for 15 days and examined for their toxic effects on the morphology of blood cells. Because these pesticides are accepted as environmental pollutants, in order to determine sublethal toxic effects in rats that were exposed to these types of pesticides, histological methods are carried out in laboratory studies.

In the microscopical studies, some histopathological changes were observed in blood cells of rats. An increase of immature red blood cells was observed due to environmental pollution. The ovoid shape seen in normal red blood cells was totally transformed and a marked increase of degenerated red blood cells is obvious. Dense and compact lymphocytes were observed instead of the small and spheric lymphocytes of the normal lymph. These studies showed that erythrocyte cells have structural defects, degenerative cell size and surface shapes and also discomformity on membrane surfaces in rats administered endosulfan and malathion. It was also observed that these pathological defects are related to dose increments.

It has been demonstrated that the disconformity of leukocyte membranes are more pronounced when compared to erytrocyte membranes (Fig. 2, 4, 5). It has been shown that the blood cells of rats in groups III and IV (Fig. 3 and 5) were more damaged than the those in groups I and II (Fig. 2 and 4). Furthermore, it has been demonstrated that the number of platelets of rats in group II and IV increased (Fig. 3 and 4). It was also observed that the blood cells (erythrocytes and leukocytes) of rats in control group (Group V) has normal histological appearance.

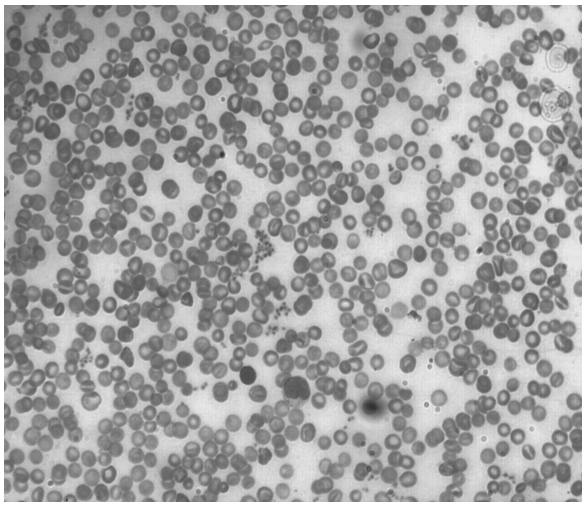

Figure 1. Photomicrographs of normal blood cells from control group of rats (Group V). X40 Giemsa stain.

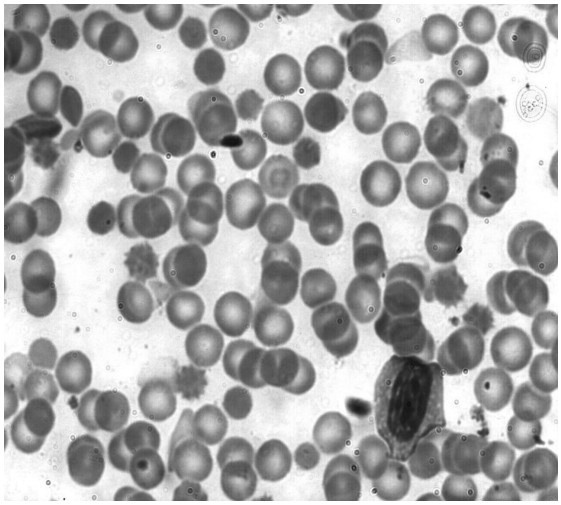

Figure 2. Photomicrographs of blood cells of low dose endosulfan group of rats (Group I). Aglutinations have been observed in red blood cells. X100 Giemsa stain.

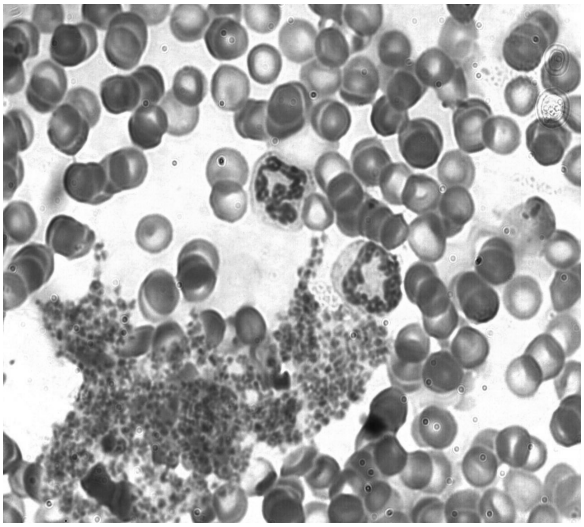

Figure 3. Photomicrographs of blood cells of high dose endosulfan group of rats (Group II). Dense and compact lymphocytes have been observed. Aglutinations have been observed in red blood cells. X X100 Giemsa stain.

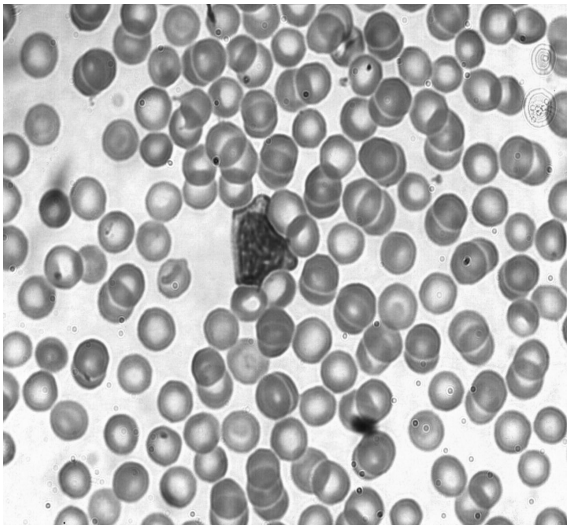

Figure 4. Photomicrographs of blood cells of low dose malathion group of rats.(Group III). Aglutinations have been observed in red blood cells. X X100 Giemsa stain.

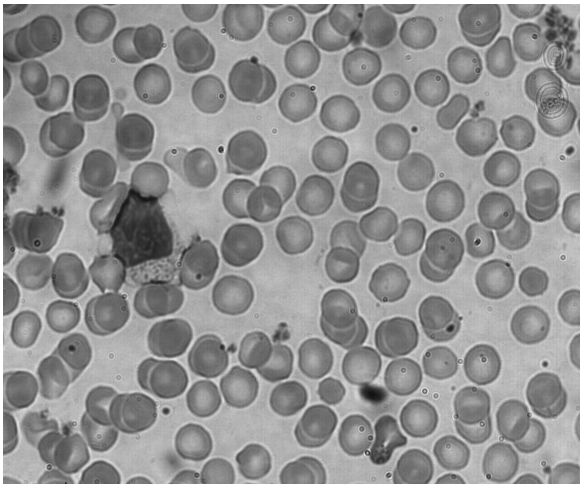

Figure 5. Photomicrographs of blood cells of high dose malathion group of rats (Group IV). Dense and compact lymphocytes have been observed. X100 Giemsa stain.

DISCUSSION

Pesticides are used extensively in agriculture and their residues have affected the environment adversely. The use of such biologically active compounds poses potential problems of toxicity among these who manufacture, formulate or use these compounds. In the present investigation, it has been observed that both endosulfan and malathion caused histopathological changes in blood cells of the Wistar albino rats. Our knowledge about normal compositions of physiological functions and biochemical processes in rats are being improved increasingly. Most toxic chemicals are metabolized in the liver and these processes may cause liver injuries [2]. Normal values of blood and tissues for many species are being examined. The effect of environmental pollution can be clearly demonstrated by histological observations of peripheral blood preparations. Structural defects may be seen in blood cells that are exposed to various pesticide types. The deleterious effects of pesticides on blood cells have been shown by animal studies. Insecticides with organophosphorus affect the normal functions of erythrocytes by damaging the membrane properties of these cells. Moreover, some pesticides may lead to damage to the size and surface shapes of erythrocytes [6]. In 1992, Nikinmaa showed morphologically damaged erythrocyte formations in wistar albino rats exposed to two different pesticide types, by using microscopic examinations of blood. They suggested that toxic materials directly or indirectly damage the membrane structure, ion permeability and cell metabolism of erythrocytes. In our study, supportive data has been obtained in accordance with this study [7].

Erythrocytes in fish exposed to several toxic materials usually have membrane defined as echinocyte (erythrocytes with spicule), and cells may lose their oval structure and may be fusiform or spherical in appearance [8]. As lymphocytes and neutrophils are involved in the natural defense mechanism in the body, they increase with the pesticide toxicity. It was considered that obtained data may be related to the immune system [1]. The major cell type of the immune system are lymphocytes, produced in large numbers during the early development stages, and recognize the antigens. But the number of these cells differs over time [9,10,11].

Monocytes, neutrophils, eosinophils and basophils also have an important role in the natural defence system. It was reported that when nuvacron and furadan are administered intraperitoneally to mice, the effects on bone marrow appears as suppression in erythroid cells and an increase in myeloid cells and also splenomegaly. Therefore, damage to blood cell structure is reported as anticipated evidence [12]. We have also observed subacute toxicity in rats. However, there is no study examining the normal blood histology of wistar albino species. Hence, we believe that this trial will cast a new light on further studies. Contributing to its popularity is its relatively low acute mammalian toxicity [10,13,14]. Farrag reported that treating rats with 1/10 LD50 of lufenuron and profenos caused significant changes in blood contents and some chemical parameters of treated rats without a return to normal levels at the end of the recovery period (30 days) [15].

However, like other pesticides that have been found to cause irreparable damage to human and environmental health, malathion may pose a greater risk than the product label would lead one to believe [11]. For example, permethrin, another synthetic pyrethroid, caused concentration-dependent increase in both apoptotic and necrotic cell death in thymocytes [16]. Shown to be mutagenic, a possible carcinogen, implicated in vision loss, causing myriad negative health effects in human and non-target organisms and containing highly toxic impurities, malathion has a legacy of serious problems [4,11,14,17,18].

In conclusion, deformations were observed in nucleus and cytoplasm of blood cells in rats due to different doses of endosulfan and malathion. It was shown that erythrocyte cells have structural defects, degenerative cell size and surface shapes and discomformity on membrane surfaces in rats administered endosulfan and malathion in different doses was also shown. It was also observed that these pathological defects are related to dose increments. Based on our results and literature data, we suggest that endosulfan and malathion usage might cause hazardous effects in various levels to non-target organisms, including human beings.

Acknowledgements

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