

Ultrastructural Effects of Ultraviolet Radiation on the Spleen of Mole Rats

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Abstract

The spleen is the part of immune system in humans and other mammals taking part in the development and maintenance of the cellular and immune responses. The purpose of this study was to elucidate the possible toxicological effects of ultraviolet C (UVC) radiation on the ultrastructure of spleen tissues of mole rats (*Spalax leucodon*). Thirteen mole rats were caught from the nature and divided into control and experimental groups. Control group did not receive any radiation. The other groups were irradiated with UVC radiation for 14 and 60 days. After the euthanasia, the spleen samples were taken, dissected out, fixed and then examined on transmission electron microscope. Depending on the radiation exposure, a number of ultrastructural changes were observed in the experimental groups. These changes were destruction on lymphocytes, reticular cells, macrophages and dilatation in mitochondria. After 14 days of UVC radiation exposure, significant changes were observed in different structural components of the spleen. But, the most destructive changes occurred on the spleen tissue after 60 days of radiation exposure. These findings clearly indicated the ultrastructural effects of UVC radiation on the spleen cells in an exposure period dependent manner.

Keywords: Electron microscope; Mole rats; Spleen; UV radiation.

Introduction

All living organisms are under the negative effects of radiation coming from the sun and other resources. More than 90% of this radiation comes from natural sources like the sun, cosmic rays, terrestrial sources, air, food and water etc. Ultraviolet C radiation (UVC) is the part of solar radiation that has more detrimental effects on living organisms than the others [1, 2]. But, the majority of this

radiation is filtered by the ozone (O₃) layer in the stratosphere. Due to releasing of the chemical substances (CFC, CFCl₃ and CF₂Cl₃), green gases and cosmetic sprays into the atmosphere, the thickness of ozone layer has reduced in recent years. Because of this reduction in the layer, it is estimated that skin cancer, cataract and immune deficiency syndrome cases will increase in the near future [1, 3, 4], and it will have an impact on the internal organs of living beings.

The immune system is one of the most sensitive systems of the organisms which quickly reacts to intracellular and external effects. The spleen is a vital haemopoietic and immune organ in these organisms, taking part in the development and maintenance of the cellular and immune responses, the innate and adaptive immunity, quantitative and qualitative content of blood cells and other lymphoid organs[5-7].

Despite the presence of the numerous researches have been done on the structure of the spleen under the effects of external factors[8-11], the effects of UVC radiation on the spleen structure have not been studied despite the fact that it comprises the main component of external components.

Mole rats (*Spalaxleucodon* Nordmann) are fossorial rodents which live in underground galleries. In normal conditions, they are not under the effects of solar radiation during their lives. For this reason, the present study was performed to evaluate the effects of UVC radiation on the spleen tissue of mole rats. Therefore, they were exposed to an artificially produced UVC radiation in the laboratory, and the findings were compared to the control group results.

Materials and Methods

Specimen Collection: Thirteen mole rats (*Spalaxleucodon*) of both sexes, weighing 200-300g were caught within the rural

areas of Ankara, Turkey. They were housed separately under the standard laboratory conditions at 21±2°C in special cages called “terrarium”. They were fed with carrots, potato and plant roots, and no special diet was given.

Radiation Source: A “Mazda TG” ultraviolet lamp in 30 Watt powers and in 90 cm length was placed to the cover of the terrarium. The intensity of the UV radiation emitted from the lamp was measured to be 254 nm in wavelength and the energy in one second was found to be 0.0014 joule/cm².

Experimental Design: After certain days of acclimatization, the mole rats were divided into three groups. Before the experiment, their dorsal parts were shaved. Group I was separated as the control and not receive any radiation. Taking into account of sunlight period, the other groups were irradiated with artificial UVC radiation for 8 hours daily (between 08.00-17.00 hours). A feeding interval was given at midday for 1 hour. A timer was used to standardize the radiation exposure times. While group II was irradiated for 14 days, group III was irradiated for 60 days. Experimental groups, exposure times and total dosages of the study groups were enlisted in Table 1.

Table 1: Experiment groups, exposure times and total dosages applied.

Experimental groups	Number of mole rats in each groups	Exposure times Day (d)	Total dosage (joule/cm ²)
Group I (Control)	5	No radiation	0,00
Group II	4	14	564,48
Group III	4	60	2.547,70

Preparation of the Samples for Transmission Electron Microscope: Before the experiment (for control) and at the end of 14 and 60 days, under the anaesthesia, the mole rats were sacrificed, the spleen tissue was rapidly removed and dissected out carefully and utilized for electron microscopic examinations.

The spleen tissues were cut into small pieces, fixed in 2.5% glutaraldehyde and in 0.1 M phosphate buffer (pH 7.2). Specimens were washed in buffer and post-fixed in 1% osmium tetroxide, dehydrated through graded concentrations of ethanol, cleared in propylene oxide and embedded in Araldite. Ultrathin sections were cut with ultramicrotome, mounted on copper grids and double stained with saturated uranyl acetate for 20 min. and lead citrate for 10 min. Sections were examined with Jeol JEM 100 CX-II electron microscope.

All experiments were carried out in accordance with the Ankara University guidelines for the care of experimental animals. Also, guiding principles for experimental procedures found in Declaration of Helsinki of the World Medical Association regarding animal experimentation were followed

in the study. The study was approved by Animal Experiments Ethical Board of University.

Results

Electron Microscopic Results of the Control Group:

Electron microscopic examination of the white pulp showed that the normal animal spleen cells were composed predominantly of lymphocytes and reticular cells. The lymphocytes vary in size and they are often difficult to differentiate from the reticular cells. But, in general, they are round and/or oval and characterized by a high nucleoplasmic ratio. Lymphocytes possess large numbers of ribosomes distributed throughout the cytoplasmic matrix that give the cytoplasm a stippled appearance. The cytoplasm is very sparse and the Golgi apparatus is relatively small and inconspicuous. These cells have a little rough endoplasmic reticulum (rER) and a few mitochondria (Figures 1a, 1b and 1c).

The reticular cells are extremely polymorphic. However, they are usually larger and they have irregular shapes. Some of them are similar to lymphocytes. They have less scattered heterochromatin and pale cytoplasm containing rough endoplasmic reticulum (rER), mitochondria, Golgi apparatus and free ribosomes. Also these cells show cytoplasmic processes towards the lymphocytes. The most distinctive feature of the reticular cell is its relationship to the reticular fibers. These cells can be distinguished from the lymphocytes by the relatively small amount of condensed chromatin in the nucleus and fibrillar structures in their cytoplasm. Also these cells are distinguished from the macrophages by its relative lack of lysosomal granules (Figures 1a, 1b, 1c and 1d).

The macrophage cells, virtually identical to reticular cells, but contain phagocytic debris (material) are

found in the white pulp. But, these cells are also found in the red pulp (Figures 1a, 1b and 1d).

The red pulp is comprised of venous sinuses (VS) and the splenic cords of Billroth, the tissue which lies between the sinuses. The sinuses make a complex series of anastomosing, tortuous sinuses which vary in size from small channels to large vascular pathways. Within these parts, there are mainly reticular cells, macrophages and fibroblasts. But, the reticular cells, leucocytes, platelets and red blood cells are also present. The sinuses of the red pulp are lined by endothelial cells. But, their nuclei are not always present in cells (Figures 1c and 1d).

The most common cells in the red pulp are erythrocytes. These cells are present in great number in the sinuses and in the cords. These cells include neutrophils, eosinophils, lymphocytes, reticulocytes, reticular cells, monocytes and platelets (Figures 1c and 1d).

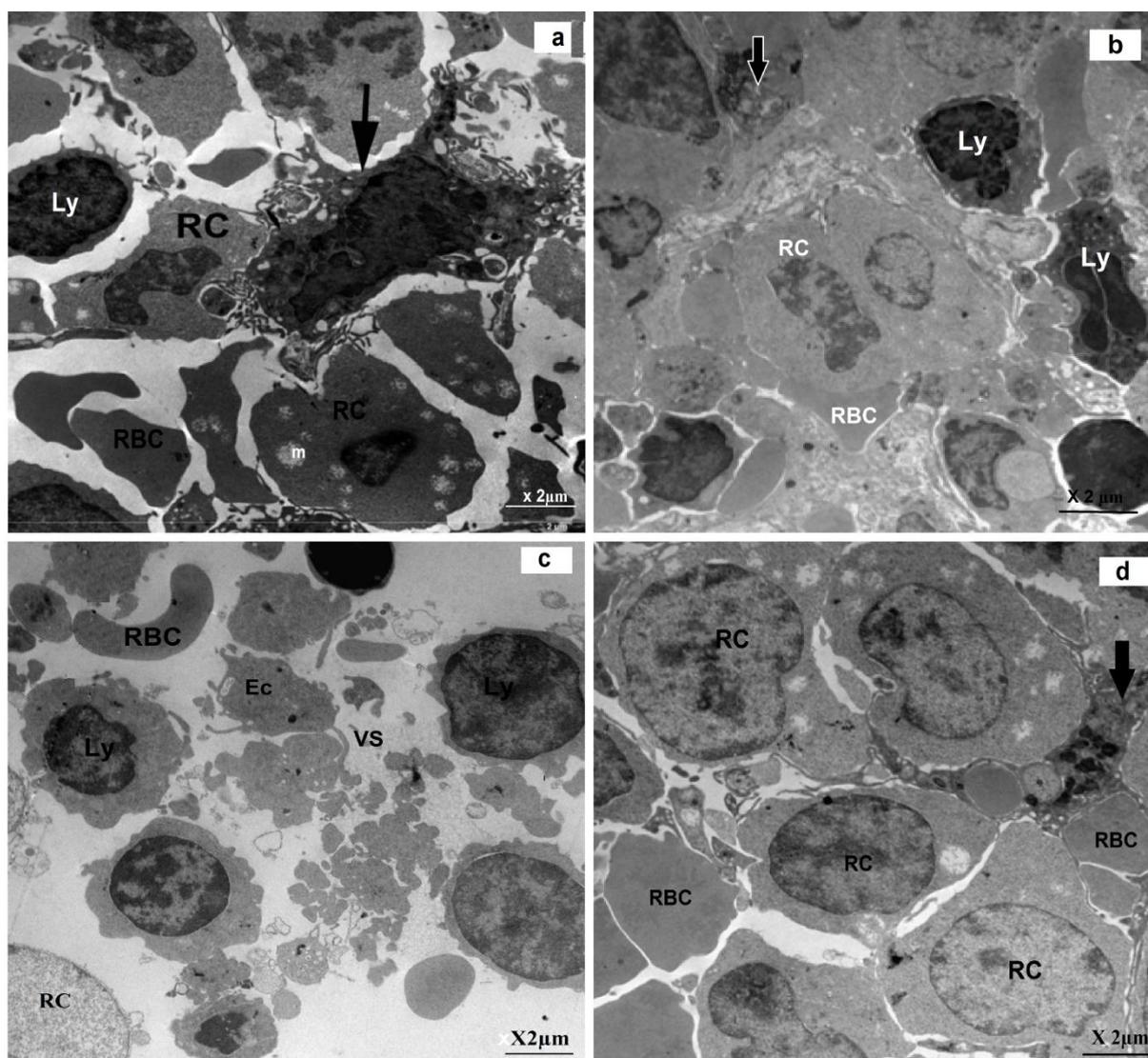


Figure 1: Electron micrographs of control group of mole rats spleen showing the lymphocytes (Ly), reticular cells (RC) and macrophages (arrow) in white and red pulps. Red blood cells (RBC), endothelial cells (EC) and venous sinuses (VS) in red pulp. Notice figure a and b were taken from the white pulp, figure c and d were taken from the red pulp.

Spleen Irradiated with UVC Radiation for 14 and 60 Days:

The experimental groups showed that after 14 and 60 days of UVC radiation exposure, significant changes were observed in all structural components of the spleen. In spleen samples there are small and medium lymphocytes and reticular cells having signs of destructive changes, irregular nuclei, oedematous cytoplasm and almost devoid of organelles.

After 14 days of exposure to UVC radiation, the white pulp was seriously destroyed. A severe damage on nucleus and cytoplasmic organelles were observed in the

lymphocytes and reticular cells. Heterochromatines were scattered throughout the nucleoplasm and margined along the nuclear envelope. In the cytoplasm of these cells, the mitochondria were vacuolated and the rough endoplasmic reticulum (rER) were dilated. Some parts of cytoplasm gained a vacuolated appearance. The macrophages were destroyed and contain a lot of electron dense materials and phagocytic debris (Figures 2a, 2b and 2c). In the red pulp, the lymphocytes, reticular cells and the other cells were destroyed and intracellular spaces were vacuolated. Also, the mitochondria in these cells were vacuolated (Figure 2d).

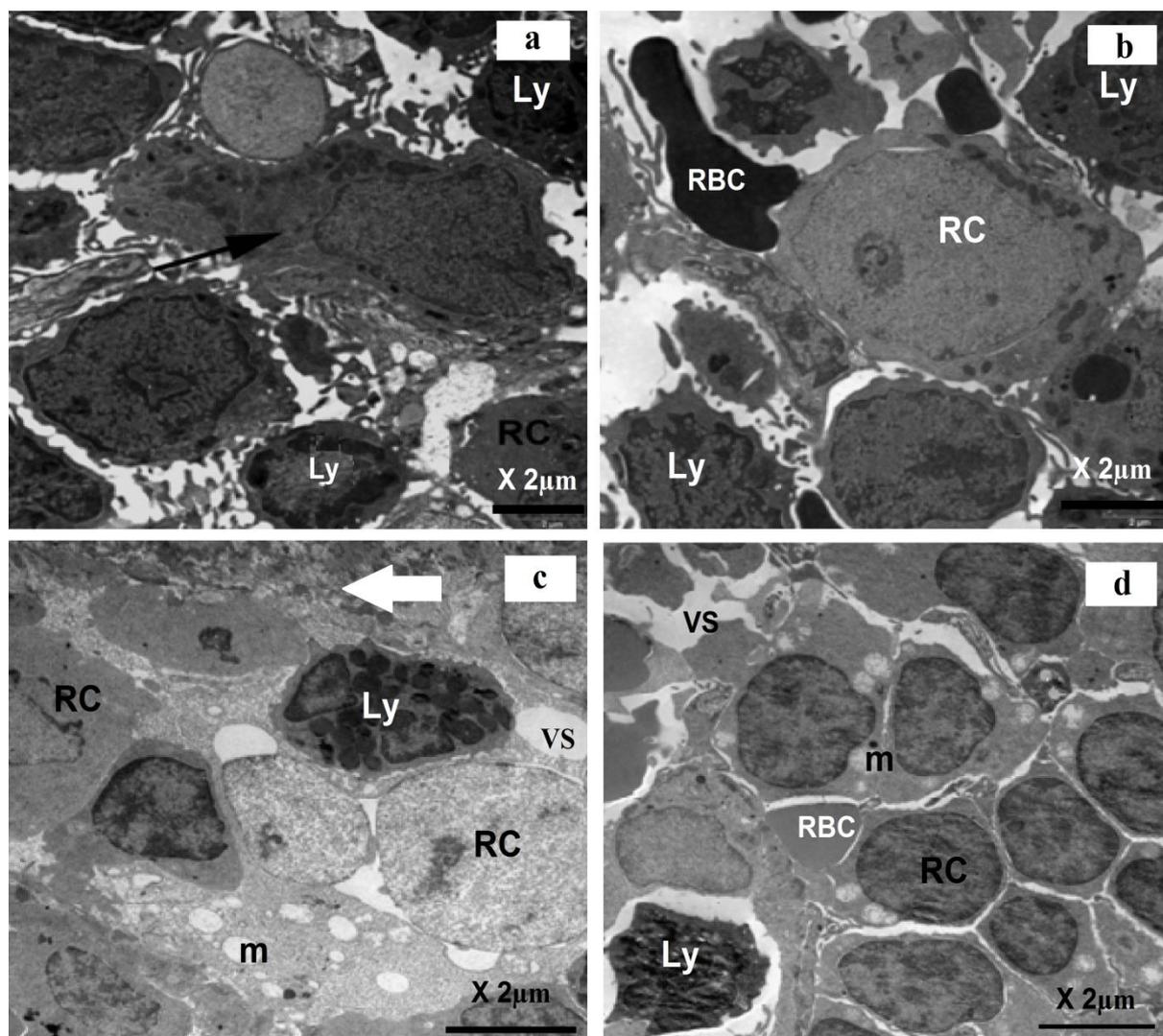


Figure 2: Electron micrographs of spleen sections of mole rats irradiated with UVC for 14 days. Anastomosing in lymphocytes [1] and reticular cells (RC), destruction in macrophage (arrow), vacuolation in mitochondria (m) and intracellular spaces. Venous sinus (VS), Red blood cells (RBC). Notice figures a, b and c were taken from white pulp, figure d was taken from red pulp.

After 60 days of exposure to UVC radiation, apoptosis in lymphocytes, reticular cells and macrophages were observed. Anastomosing in lymphocytes and reticular cells also occurred. The nuclei of these cells were changed and some of them were disintegrated. In lymphocytes, the nuclei lost normal appearance and chromatin was condensed around the nucleus border (Figures 3a and 3b). The macrophage cells were seriously destroyed and had some

phagocytic materials (Figures 3a and 3c). In reticular cells nucleoplasm had a pale appearance with fine granules. The nuclei of these cells were lost and the mitochondria were vacuolized (Figures 3a-3d). In the red pulp, the lymphocytes, reticular cells and the other cells were seriously destroyed and vacuolation was increased (Figures 3c and 3d).

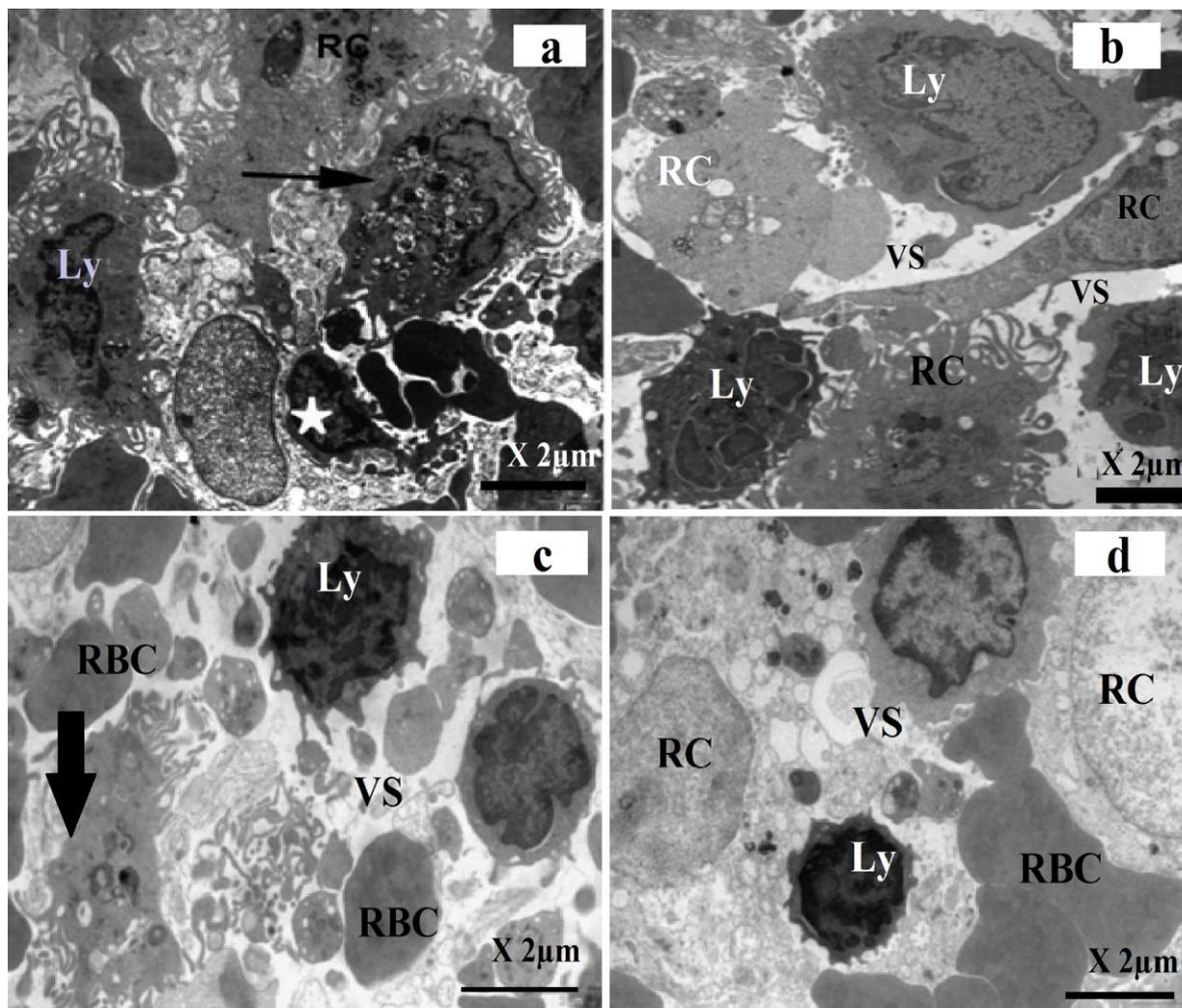


Figure 3: Electron micrographs of spleen sections of mole rats irradiated with UVC for 60 days. Anastomosing in lymphocytes (Ly); destruction in reticular cells (RC), macrophages (arrow) and the other parts of the spleen cells. Vacuolation in mitochondria (m) and between the cells. The red pulp also seriously was destroyed. Notice figures a, b and c were taken from white pulp, figure d was taken from red pulp.

Discussion

The spleen is a vital hematopoietic and immune organ in humans and other mammals [5, 12, 13]. The immunological function of spleen is mainly carried out by white and red pulps, which consist of aggregates of lymphoid tissues [11]. The injury of spleen can cause dysfunction of immune system and make one susceptible to infections and other diseases.

Living organisms have well protective and effective cellular repair mechanisms against radiation coming from the sun and other resources. However, the excessive radiation exposure may cause damage on the cells, immune system and blood cells [14, 15]. The pathological effects of radiation begin immediately after radiation exposure, but the histological effects may not become apparent for weeks, months, or even years after exposure [16].

The destruction symptoms are developed depending on the exposal period and the dosage, and they may be developed gradually or suddenly [15, 17, 18]. While the high dose of radiation destroys the cell structure, the low dose of radiation is no longer considered to be as harmful as once thought; because the pathological effects can be prevented by some antioxidants in cells [19].

Apoptosis is the process of programmed cell death occurrence on multicellular organisms. It is well known that excessive radiation induce cellular injury due to the harmful effects of the free radicals which play a key role occurring apoptosis on the cells [20, 21]. Such biochemical substances lead to structural changes on the cells and cell death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation and global mRNA decays [21 - 26].

In this study, significant alterations were detected in white and red pulps microscopically after 14 and 60 days of UV radiation exposure. These changes were mostly seen on lymphocytes, reticular cells and macrophages. When it comes to the period of radiations applied, respectively; lymphocytes, reticular cells and macrophages had a necrotic appearance after 14 days radiation exposure. In some cells, dilatation was encountered between the cells, vacuolization appeared in mitochondria and destruction also occurred in the membrane structure of cells and nuclei. These results were consistent with some studies done with different radiation [23, 24]. Such degenerative changes that occur in cytoplasm and organelles resulting in significant functions in each phase of radiation in order that cell could carry on its crucial activities would certainly decrease its process and distort its structure.

More advanced pathological effects were encountered in white and red pulps after 60 days radiation

exposure. Due to radiation dose and exposure time, destruction increased in lymphocytes, reticular cells and macrophages. Most of these cells borders disappeared and vacuolization in mitochondria increased. Macrophage activities also increased as a possible response to radiation. The macrophage activity may be related with the increased apoptosis phenomena in the spleen of mole rats after exposure to UV radiation. Similar results were encountered in some studies done before [25].

High activity of apoptosis in the white and red pulps of the spleen of mole rats after 14 and 60 days exposure may be occurred due to radiation dose and exposure period. According to the literature, excessive radiation increased the apoptosis on the lymphoid and reticular cells [27-30].

Conclusion

The present results indicated the ultraviolet radiation effects on the spleen cells of mole rats. The effects on the spleen cells increased *depending on the radiation dose and* application time. The longer exposure to ultraviolet radiation shows more harmful effects on living cells. Loss in ozone layer will result with increased ultraviolet exposure that will cause skin cancer, cataract, and immune deficiency and impairs on cell structures. There is a need for more other studies in order to determine the relation between the changes that occur in the ultrastructure of the cell and the radiation exposure on the cells.

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