

## HISTOPATHOLOGICAL CHANGES IN SPLEEN OF MOUSE AFTER RADIATION EXPOSURE

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### ABSTRACT

*Small amount of radioactive material released in the environment from coal and nuclear power plants and also from nuclear explosions are also source of radiation exposure to man. Exposure to high doses of radiation can cause nausea and vomiting within few hours whereas low levels of radiation exposure doesn't cause immediate health effects but can increase cancer risks. Ionizing radiation can cause tissue damage by changing the chemical properties of molecules. It produces free radicals which are chemically very active and damage the genetic material of a living cell. The present study was therefore designed to investigate the histopathological changes in spleen of Swiss albino mice after radiation exposure. For the experiment, adult healthy male Swiss albino mice were irradiated at the dose rate ranging from 0.97 Gy/min. to 1.97 Gy/min. The dose was calculated at mid-point by multiplying dose rate and tissue air ratio. Animals were exposed to sublethal dose of gamma radiation from Co<sup>60</sup> source. Five animals from each group were autopsied by cervical dislocation at each post-interval of 1, 2, 4, 7, 10, 14, and 28 days. Before autopsy the weight of the animals was also recorded. Five normal mice were also autopsied. After autopsy spleen was taken out, weighed on mono pan electric balance. After recording weight, tissue was fixed in Bouin's fluid for 24 hours for histological studies. Spleen is a radiosensitive tissue and shows reduction in body weight and organ weight ratio reaching minimum till day 10 after radiation exposure. Loss of splenic weight was mainly due to cellular damage, loss of lymphocytes, mitosis and circulatory and humoral disturbances. Most striking histopathological change in the spleen in the present study was the rapid death of lymphocytes. Decrease in the total cell population in the present study may be due to direct killing of small lymphocytes by radiation and due to death of cells in their attempt to divide. During later intervals, the new germinal centers were gradually repopulated and lymphocyte cap begins to surround them. At the end of the experiment, although spleen represented all the normal cell types however, recovery was not complete as far as cell population and cell arrangement was considered.*

**Keywords:** Radiation, Spleen, Lymphocytes.

### Introduction

Radiation is the natural part of our environment and every living being on the earth is exposed to this natural background ionizing radiation. Small amount of radioactive material released in the environment from coal and nuclear power plants and also from nuclear explosions are also source of radiation exposure to man. Increasing use of ionizing radiation for diagnostic as well as therapeutic purpose has drawn the attention of many radiobiologists towards undesired adverse effects of such exposures. Exposure to high doses of radiation can cause nausea and vomiting within few hours whereas low levels of radiation exposure doesn't cause immediate health effects but can increase cancer risks. The biological effect of these exposures varies with the type, energy and dose of radiation. Ionizing radiation has sufficient energy to liberate electrons from atoms and thereby ionizing them which further damage the genetic material of a living cell, however, our body cells are efficient enough to repair this damage up to certain levels. One of the well-known consequences of radiation exposure is the changes in blood cell counts. Due to the high sensitivity of blood and blood forming organs to ionizing radiation variations in blood cell count is still considered the most sensitive biological evidences of several disorders or diseases. Changes found in the circulating blood are primarily due to the effect on haematopoietic tissues (1 & 2). A very small dose of radiation to a blood forming organ causes an arrest of the haematopoiesis with changes in peripheral blood.

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Spleen is highly radiosensitive haematopoietic organ and effect of ionizing radiation on the spleen was first noticed in 1903 (3). Ionizing radiation can cause tissue damage by changing the chemical properties of molecules. It produces free radicals which are chemically very active. Induction of DNA damage and apoptotic cell death in the spleen cells has also been reported by many scientists (4). Lack of data regarding haematological effects of spleen irradiation the present study was therefore designed to investigate the histopathological changes in spleen of Swiss albino mice after radiation exposure.

### Materials and Methods

For the experiment, adult healthy male Swiss albino mice were irradiated at the dose rate ranging from 0.97 Gy/min. to 1.97 Gy/min. The dose was calculated at mid-point by multiplying dose rate and tissue air ratio. Animals were exposed to sublethal dose of gamma radiation from Co<sup>60</sup> source.

For the present study, animals were divided into following two groups:

**Group I** – Sham-irradiated

**Group II**- Irradiated animals (5.0 Gy)

Five animals from each group were autopsied by cervical dislocation at each post-interval of 1, 2, 4, 7, 10, 14, and 28 days. Before autopsy the weight of the animals was also recorded. Five normal mice were also autopsied. After autopsy spleen was taken out, weighed on mono pan electric balance. After recording weight, tissue was fixed in Bouin's fluid for 24 hours for histological studies.

Following studies were taken into consideration:

- **Organo- Somatic Index**- Average weight of both tissue and animals were recorded. The weight of the tissue was calculated per 100 gm. body weight and expressed as organo-somatic index.

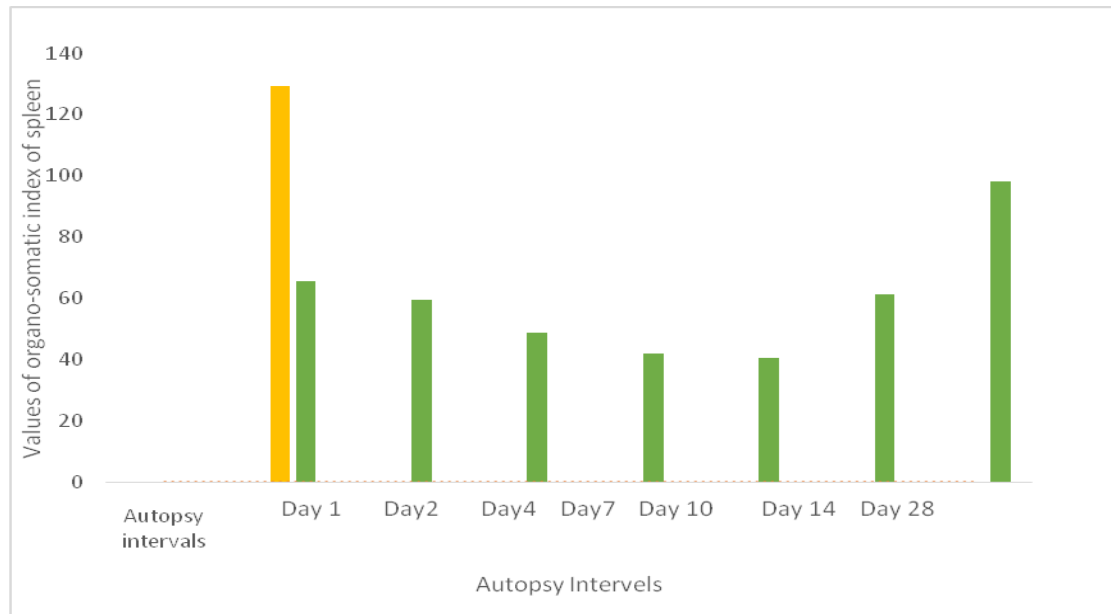
**Organo-Somatic index** =  $\frac{\text{Average tissue weight of animal}}{\text{Body weight of the same animal}}$   
(mg/ 100 gm body weight)

### Histopathological Studies

The tissue was fixed in Bouin's fluid, dehydrated and embedded in paraffin wax. Transverse sections were cut at 5  $\mu$  from the middle part of the tissue and stained with Harri'shaematoxin-eosin stain for histopathological studies.

### Results and Discussion

The changes in the values of organo-somatic index of spleen of mouse after irradiation is expressed in the histogram-1



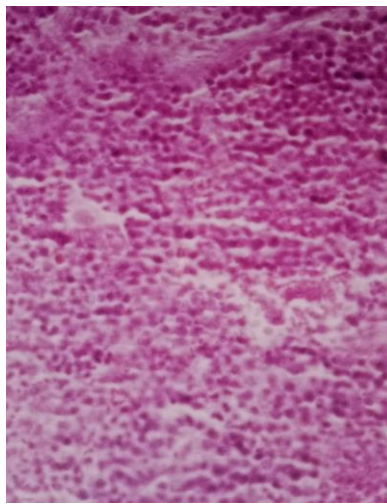
Group-1 ■

Group-2 ■

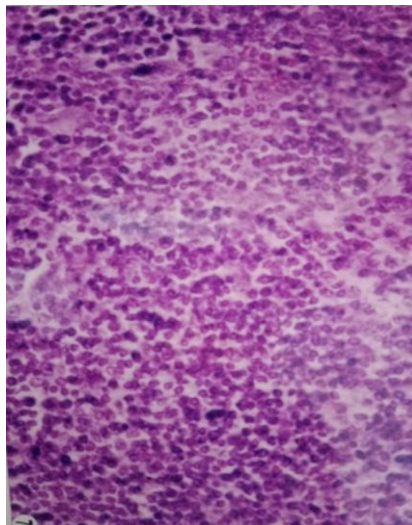
Spleen is a radiosensitive tissue and shows reduction in body weight and organ weight ratio reaching minimum till day 10 after radiation exposure in the present investigation and increasing thereafter.

Loss of splenic weight was mainly due to cellular damage, loss of lymphocytes, mitosis and circulatory and humoral disturbances. Decrease in the volume of spleen has also been reported (5). Loss of weight was directly proportional to the exposure dose (6) whereas increase in the organo-somatic index during the later intervals was brought about by hyperaemia and extramedullary diffuse haematopoiesis (7). Spleen showed pronounced recovery and the mitotic index in the regenerating areas of the active pulps of spleen reaches its maximum, a few days before the maximum increase in the spleen weight (17 days) and remains at about twice the normal value during the period of compensatory weight increase (8).

Following histopathological changes were observed after irradiation as compared to the spleen of sham-irradiated mice.



**Fig. 1: Photomicrograph of Spleen after 2 days of Exposure Showing the Tissue Strewn with Cellular Debris**



**Fig.2: Photomicrograph of Spleen after 14 days of Exposure showing a Large Number of Mitotic Figures Especially in white Pulp and Hemosiderin Pigment in Red Pulp**

The effects of irradiation on the concentration of circulating peripheral blood cells are based considerably on the radiosensitivity of the precursor cells in the haematopoietic tissue with respect to both inhibition of mitotic process and cell death with consequent hypoplasia or aplasia of the haematopoietic tissue. The haematopoietic tissue with mitotic potentials are more sensitive consisting of pools of mature dividing cells. In the steady state, the loss of mature cells from the blood is balanced by the production of new cells. Irradiation disturbs this steady-state cell renewal system.

Most striking histopathological change in the spleen in the present study was the rapid death of lymphocytes. There was a drastic reduction in the number of small lymphocytes on day 1. Radiation-induced destruction of lymphocytes has also been reported (9). In days 2 and 4, large number of macrophages loaded with nuclear debris were observed. Most of the debris was cleared by day 4 and the tissue became depopulated by day 7, degenerating cells with crenated nuclei were present. The red pulp was reduced to reticular cells and red blood cells. The presence of haemosiderin pigment was increased in the red pulp as a result of destruction of circulating erythrocytes (10,11).

Decrease in the total cell population in the present study may be due to direct killing of small lymphocytes by radiation and due to death of cells in their attempt to divide which is also supported by many researchers and scientists (12,13). There is a significant correlation between the decrease in peripheral lymphocytes and spleen irradiation dose (14). During later intervals, the new germinal centers were gradually repopulated and lymphocyte cap begins to surround them. After two weeks of exposure, spleen showed active areas of ectopic myelopoiesis also supported by the scientist (15). At the end of the experiment, although spleen represented all the normal cell types however, recovery was not complete as far as cell population and cell arrangement was considered. Radiation may cause long-term splenic dysfunction (16).

### Conclusion

- Decrease in organo-somatic index of spleen during the early intervals was probably due to the death of lymphocytes and their removal from the tissue by active phagocytosis.
- Histopathological studies revealed that radiation-induced damage increased with the increase in dose of radiation.

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