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Research Article

Histopathological Changes in the Spleen of Swiss albino Mice after the Combined Exposure of Radiation and Cadmium

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A B S T R A C T

With the technological advancement and diversification of industries, combined with specialization in all fields, the volume and complexity of metals is also increasing day by day. Interaction of metals with other agents is an important aspect as both can interact in a "synergistic" or "additive synergistic" manner, further aggravating the situation. In the present study, combined effect of radiation and cadmium on spleen of mice has been investigated. For the experiment, adult, healthy male Swiss albino mice were exposed to different doses of radiation and also fed with the aqueous solution of $CdCl_2$ which was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of glass distilled water, thus giving a concentration of 20 ppm and then administered orally *ad libitum* in drinking water continuously, till the end of the experiment. Animals were autopsied by cervical dislocation at each post- interval of 1, 2, 4, 7, 10, 14 and 28 days. Spleen was taken out, weighed, fixed in Bouin's fluid, dehydrated and embedded in paraffin wax. Transverse sections were cut at 5µ from middle part of the tissue and stained with Harri's haematoxylin-eosin stain for histopathological studies. Pathological changes after combined exposure in the present investigation depends upon the total dose of radiation provided i.e. higher the dose, higher the damage. Most striking histopathological change in the spleen was loss of lymphoid structure, inflammation, fibrous tissue proliferation, pyknosis, necrosis, karyolysis, karyorrhexis.

Keyword: Synergistic; Pyknosis; Necrosis; Karyolysis; Karyorrhexis; Inflammation.

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INTRODUCTION

All living organisms are susceptible to environmental changes and none can escape from the hazardous effects of exposure to pollutants including radioisotopes and ionizing radiations. Increasing use of ionizing radiation for diagnostic as well as therapeutic purposes has drawn the attention of many radiobiologists towards the undesired side effects of such exposures.

Since there is a constant release of trace elements in unnaturally higher concentrations and often in unusual physio-chemical state, the fear of them being hazardous to human health is now an indisputable truth. Most of the pollution problems which we face today stem from the over exploitation of our natural resources and/or heedless disposal of waste materials in the environment. Once perpetuated in the environment, metals are not readily detoxified. Metallic (arsenic, Cadmium, chromium, lead and mercury) elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure[1].

Cadmium is a wide spread toxic pollutant of occupational and environmental concern because of its diverse effects; low rate of excretion from the body predominant storage in soft tissues[2].Cadmium has many applications example in batteries, pigments, plastics, metal coatings and widely used in electroplating[3]. Cadmium is adsorbed in significant quantities from cigarette smoke, food water and air contamination and is known to have numerous undesirable effects in both human and animals[4-8]. Cadmium-mediated is thought to involves multiple mechanisms, including DNA strand breakage and inhibition of DNA repair Cadmium induces p53-dependent GI/S and/or G2/M cell cycle arrest in various cell lines expressing tumor suppressor protein p53[9-10].

It is not enough to know the effects of single agent because the presence of numerous pollutants results in a very complex network of interactions which often leads to an

intensified impact as compared to a sum total of a individual effects. However, much work on Cadmium toxicity and radiation has been carried out individually on a variety of tissues of different animals including man but sufficient information is not available on the combined effect of heavy metal and radiation and the mechanism by which they exert their own toxicity and the investigation done on combined effects are not sufficient enough to confirm their deleterious effects.

Therefore, an attempt has been made to assess the combined effects of radiation and cadmium on the spleen of Swiss albino mice.

MATERIALS AND METHODS

Adult healthy male Swiss albino mice (6-8 weeks old) were taken for the experiment. Animals were fed with standard mice feed and water *ad libitum.* Occasionally tetracycline water was provided as a precaution against infections.

Co^{60} gamma radiotherapy source (Theratron) of AECL make, obtained from Canada was used to expose the animals. The animals were irradiated at the dose rate ranging from 0.97 Gy/min. to 1.97 Gy/min. The dose was calculated at midpoint by multiplying dose rate and tissue air ratio.

Along with this, the aqueous solution of Cadmium Chloride prepared by dissolving 20 mg of $CdCl_2$in 1000 ml of glass distilled water, thus giving a concentration of 20 ppm and administered orally in drinking water.

The animals for the experiment were divided into following groups:

Group I – The animals of this group were sham-irradiated and served as control (Normal).

Group II – All the animals of this group were orally fed with Cadmium Chloride solution (20ppm) *ad libitum* and also exposed to different doses of radiation and was divided in three groups on the basis of radiation dose received:-

Group II A - 1.25 Gy + $CdCl_2$

Group II B – 2.50 Gy + $CdCl_2$

Group II C – 5.0 Gy + $CdCl_2$

Three animals from each group were autopsied by cervical dislocation at each post-interval of 1, 2, 4, 7, 10, 14, and 28 days. The weight of the animals was recorded before autopsy.

Three normal mice were also autopsied.

Spleen was taken out after the autopsy and weighed on monopan–electric balance. After recording the weight, tissues were fixed in Bouin's fluid for 24 hours for histological studies.

Organo-somatic index

Average weight of the tissue was recorded. The weight of the tissue was calculated per 100 gm. body weight and expressed as organo-somatic index.

$$\text{Organo-somatic Index} = \frac{\text{Average tissue weight of animal}}{\text{Body weight of the same animal}} \text{ X100(mg/100gm body weight)}$$

Histopathological Changes:

The tissues were fixed in Bouin's fluid, dehydrated and embedded in paraffin wax. Transverse sections were cut at 5µ from the middle part of the tissue and stained with Harris haematoxylin-eosin stain for histopathological studies.

RESULTS

Organo-Somatic index

The changes in the values of organo-somatic index of spleen of mice in various experimental groups are expressed in histogram-1.

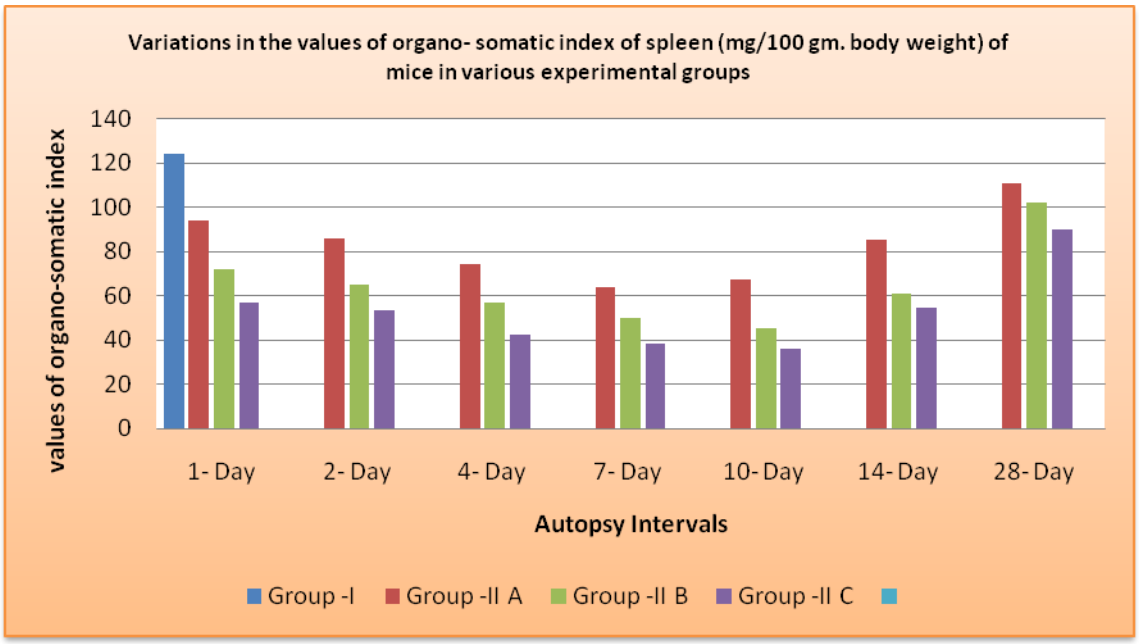


Figure: 1 Histogram of the changes in the values of organo-somatic index of spleen of mice in various experimental groups

Histopathological changes

The following histopathological changes were observed in various experimental groups as compared to the spleen of Sham-irradiated mice. (Figs.2-7). In photomicrographs we can see that higher the dose of radiation, higher the damage.

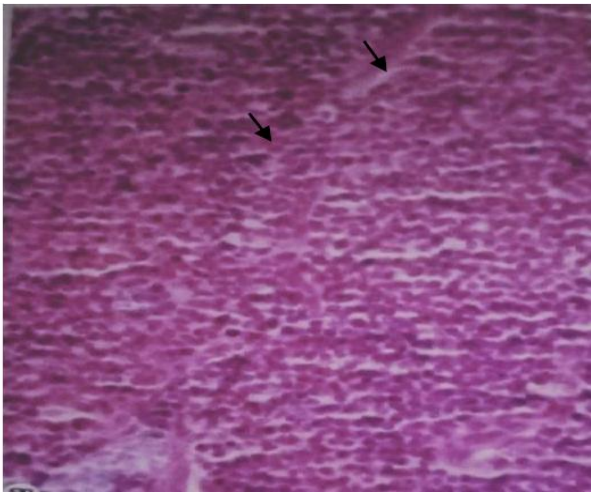
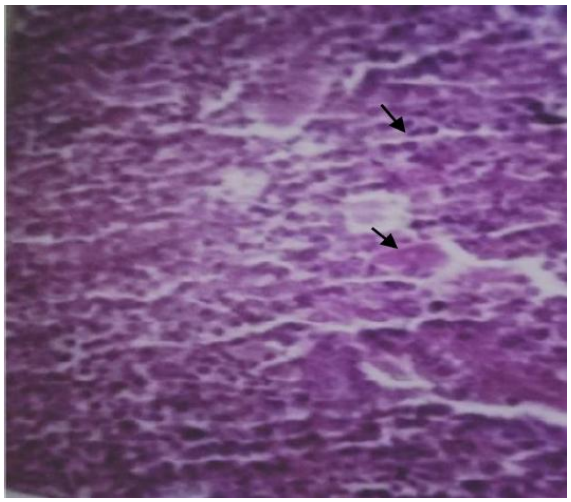


Figure-2 x 400



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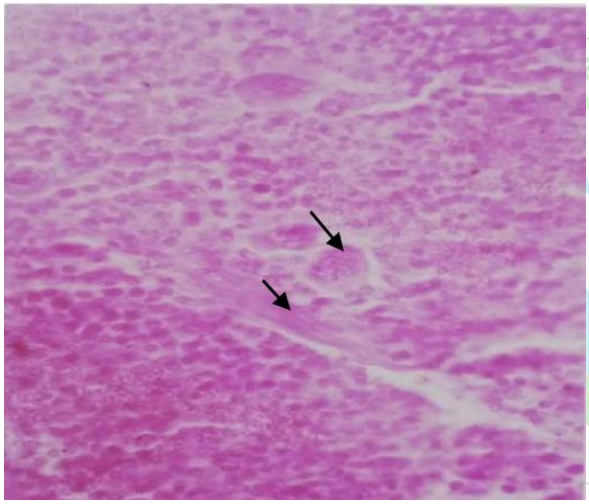

Figure-4 x 400

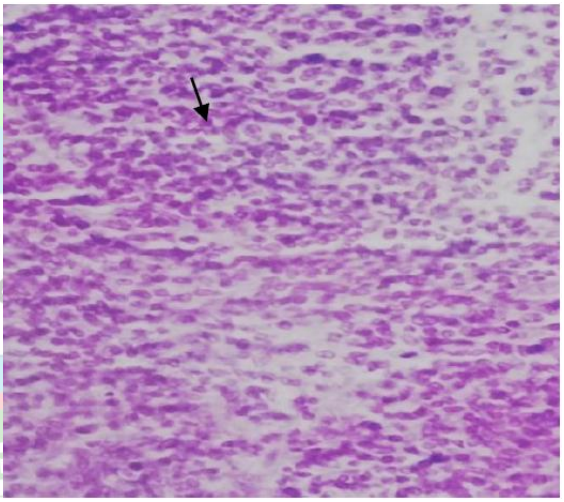

Figure-5 x 400

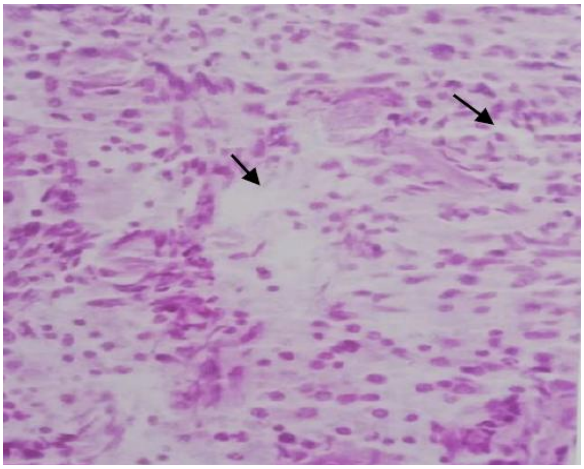

Figure-6 x 400

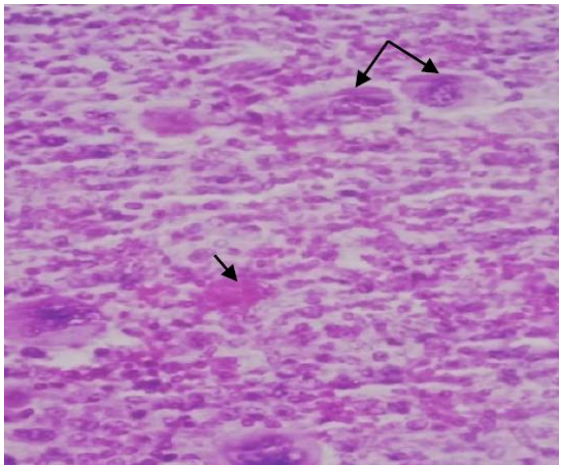

Figure-7 x 400

Figure-2. Photomicrograph of spleen of mice after 2 days of combined treatment of gamma radiation(1.25 Gy.) and $CdCl_2$ showing tissue filled with nuclear debris and fibrosis in some parts.

Figure.-3. Photomicrograph of spleen of mice after 7 days of combined treatment of gamma radiation (1.25 Gy) and $CdCl_2$ showing depopulated white pulp, presence of few megakaryocytes and number of dividing cells..

Figure.-4. Photomicrograph of spleen of mice after 4 days of combined treatment of gamma radiation (2.50 Gy) and $CdCl_2$ showing marked reduction in cell population, few megakaryocytes and tissue shows fibrosis in some regions.

Figure.-5. Photomicrograph of spleen of mice after 10 days of combined treatment of gamma radiation (2.50 Gy) and $CdCl_2$ showing increase in cellularity and active germinal centers with many dividing cells.

Figure.-6. Photomicrograph of spleen of mice after 7 days of combined treatment of gamma radiation (5.0 Gy) and $CdCl_2$ showing a severe depletion of lymphoid cells and the tissue appears as a network of fibrous tissue strands.

Figure.-7. Photomicrograph of spleen of mice after 1 4 days of combined treatment of gamma radiation (5.0 Gy) and $CdCl_2$ showing hemosiderin pigment scattered throughout the red pulp and increase in the number of megakaryocytes.

DISCUSSION

The haematopoeitic tissues with mitotic potentials are more sensitive and the life of the animal is dependent upon the continuous replacement of the mature functioning cellular elements by the mitotic cells. One of the characteristic features of radiation injury is the disturbance of this steady-state of cell renewal system. In addition to damaging DNA, ionizing radiation alters gene expression and transcription and interferes with intracellular and intercellular signaling pathways[11]. A dose-dependent change in spleen size has also been observed after chemoradiation therapy for gastric cancer[12]. A dose-dependent change in spleen volume after radiation therapy was also demonstrated[13].

Heavy metals induce oxidative stress by generating free radicals and reducing antioxidant levels.

Heavy metals also alter the confirmation of protein and DNA and inhibit their function[14]. Cadmium can cause both acute and chronic intoxications including essential hypertension, renal dysfunction[15].

In the present investigation spleen shows reduction in body weight and organ weight ratio till day 7 and increasing thereafter. The decrease was more prominent in group IIC. Loss of spleen weight was mainly due to cellular damage, loss of lymphocytes mitosis and circulatory and humoral disturbances. All the groups showed the similar pattern of decline in organo-somatic index but the decline was more severe in group IIC as compared to group II A& II B.

Pathological changes observed after the combined exposure of radiation and cadmium chloride in the present investigation depends upon the total dose of radiation provided i.e. higher the dose, higher the damage. 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. On 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 7and degenerating cells with crenated nuclei were present. The natural arrangement of cells was also disturbed, and there was no demarcation between red and white pulp. Besides these, the red pulp was reduced to reticular cells and red blood cells. At that period, the presence of hemosiderin pigment was increased in the red pulp as a result of destruction of circulating erythrocytes. 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.

Other than this, the general histopathological changes in the spleen of mice were loss of lymphoid structure, inflammation, fibrous tissue proliferation, hyperplasia of lymph follicles, pyknosis, karyolysis, karyorrhexis, necrosis, granulocytic infiltration and vacuolation. At the end of the experiment, although spleen represented all the normal cell types, however the recovery was not complete as far as cell population and cell arrangement was concerned.

In combined exposure cadmium interacts with ionizing radiation because of its high affinity for – SH and disulphide groups of proteins[16]. In addition to this, Cadmium combines with Thiol groups of cellular components, which in turn, are primarily responsible for protecting repair systems against damage caused by radiation-induced free radicals[17]. Indirect effects of radiations causes formation of free radicals which are scavenged by –SH groups, an inherent protective mechanism present in the cells. When cadmium is simultaneously administered with radiation, these –SH groups are not available for protection because cadmium binds with them. This results in DNA exposures to these free radicals and leads to increase risk during combined exposure. Deleterious reaction of these oxyradicals with biomolecules results in cellular DNA strand breakage, protein oxidation and lipid preoxidation. Both radiation and cadmium attack –SH group of proteins and reduce liver glutathione (GSH) level which is a natural cell legend providing protection to the tissues. This lower GSH level may be responsible for the biochemical alterations[18]. Both radiations and cadmium also induce damage to the cell organelles by altering membrane permeability and enzymatic activity. Cadmium inhibits the repair replication system of DNA because of its binding affinity with –SH groups of enzymes[19].

In the present study, animals exposed to radiation and cadmium simultaneously, showed severe damage indicating their "Additive Synergistic" effect.

CONCLUSION

From the present investigation following can be deduced:

- Decrease in organo-somatic index values of spleen during early intervals was probably due to the death of cells and their removal from the tissue by active phagocytosis.
- Histopathological studies revealed that combined exposure to radiation and cadmium chloride registered more pronounced changes with the increase in dose of radiation.

Thus, it can be deduced that radiation and cadmium exerts "additive synergistic" effect on haematopoeitic tissues of mice**.**

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