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Study the Effect of Gamma Rays on the Spleen and Liver of Albino Male Mice

[1]Hamzah Jasim Joudoh, [1]Nada Farhan Kadhim and [2]Amany Mohammed Al-Kaysi
[1]Department of Physics, College of Science, Mustansiriya University, Baghdad, Iraq
[2]College of Health and Medical Technology, Middle Technical University, Baghdad, Iraq

Abstract: The present experiment aimed to determine the effect of gamma ray at doses of 11.883 μSv on splenic and liver tissue of albino male mice, at the different time of exposure (4, 8, 12, 16, 21, 24, 32 days). Irradiated mice were sacrificed after inhalator anesthesia, then tissue's samples of spleen and liver were taken from all experiment's animals for histopathological study, the results of pathology showed seriously changes within splenic tissues represented by depletion of white pulp more clearly after 12 days with proliferation of megakaryocyte with infiltration of inflammatory cells out of peripheral blood vessels, spleen, also, showed depletion of lymphoid follicles after 24 days post radiation and severe destruction of spleen parenchyma with congestive blood vessels in addition to deposition of amyloid-like substances were also, observable. These depositions of amyloid-like substances appear more clearly around lymphoid follicles after 32 days irradiation. Liver tissues at early exposed time (4, 8, 12, 16 days) showed degenerative changes as; Enlargement of hepatocytes with absence of sinusoids and increase in the number of kupffer cells with infiltration of inflammatory cells in a sinusoid, also, showed large necrotic area appear surrounded by the inflammatory zone. After 21, 24, 32 days livers tissues showed focal aggregation of inflammatory cells in many sections appears as focal granuloma.

Key words: Ionizing radiation, gamma ray, spleen, liver, inflammatory cells, kupffer cells

INTRODUCTION

Humans and other living individuals can be exposed to ionizing radiation by accidental damage of nuclear power plants and by radiological or nuclear devices (Golla *et al*., 2017). It may produce cancer, loss of neural function, death and it may induce killing, mutation and chromosomal aberrations in cells (Sutherland *et al*., 2000). Ionizing radiation is known as a mutagenic and carcinogenic agent of mammals including humans, not only in high but also, lower doses and medical staff who uses radiation for therapeutic and diagnostic purposes are likely to be at risk of excessive exposure (Dobrzynska *et al*., 2014). Ionizing radiation displaces electrons in the atoms of the material through which it passes and produces charged particles, ionizing radiation can through direct interaction with the tissues the cause induce cellular damage or indirect interaction by generating free radicals and inducing inflammation (Deas *et al*., 2017). Interaction of ionizing radiation with living cells produces a variety of changes depending on kind of exposure, duration of exposure, absorbed dose, the period after exposure and also, the susceptibility in tissues (Soliman *et al*., 2015). Exposure of the body to ionizing radiation produces the Reactive Oxygen Species (ROS) which damages proteins, lipids and nucleic acids (Mohamed *et al*., 2016). Gamma rays are energetic and high-frequency electromagnetic waves (Shahid *et al*., 2015). γ-rays have no charge and it has very high ionizing energy because of their high energy, gamma photons travel at the speed of light and can cover hundreds to thousands of meters in the air before spending their energy hey can pass through many kinds of materials, including tissues (Abojassim *et al*., 2015). Ionizing radiation, including gamma radiation is characterized by high biological activity. When interacting with living cells it causes ionization of different chemical compounds and bio substrates which leads to complex reactions and processes in the cells and tissues (Tanchev *et al*., 2015). The biological effects of radiation are harmful to life. The severity of radiation effects depends on whether the energy of radiation absorbed by tissue molecules or the surrounding water. The animal body consists of 60-80% water, hence, largely, the biological effects are mainly mediated through the action of radiation on water (Maharwal *et al*., 2005). The spleen is the largest secondary immune organ in the body and is responsible for filtering the blood of foreign material and damaged old or red blood cells and for initiating immune reactions to blood-borne antigens these functions are carried out by

Corresponding Author: Hamzah Jasim Joudoh, Department of Physics, College of Science, Mustansiriya University, Baghdad, Iraq

the 2 main compartments of the spleen, the white pulp (including the marginal zone) and the red pulp (Cesta, 2006). The red pulp of the spleen removes the aged and damaged RBCs and blood-born microorganisms from the circulating blood. In this process macrophages, monocytes, megakaryocytes and granulocytes (Eosinophil's, Basophils and Neutrophils) participate actively. Monocytes reside in the red pulp cords of the spleen and are in far greater number than the total number of monocytes in circulation. They can be quickly mobilized to leave the spleen and participate in combating infections (Bala and Kaur, 2015). The spleen plays an important role in immune function by trapping and processing antigens, homing, transforming and proliferating lymphocytes and activating macrophages (Ezz, 2011). The excessive radiation exposure may cause damage to the cells, immune system and blood cells and the injury of the spleen can cause dysfunction of the immune system and make one susceptible to infections and other diseases (Periasamy *et al*., 2009). The liver is the biggest solid organ in the body, representing 5% in mice and 2% of total body weight in humans, the liver is responsible for a myriad of functions, including bile production/secretion, synthesis of serum proteins, lipids and amino acids, metabolism of carbohydrates and detoxification of xenobiotic compounds. These roles are performed primarily by hepatocytes that comprise 70-80% of the liver mass (Duncan, 2013). It was considered earlier relatively resistant to gamma radiations, the liver is an organ which suffers from direct and indirect both the types of damage, irradiation causes necrosis in the liver, all the tissues including hepatic parenchyma, reticuloendothelial tissue, arteries and capsule are affected in the liver after irradiation to high doses of gamma radiation, high doses of irradiation caused histopathological changes in the liver these include a distorted arrangement of hepatic cords, wider and thinner sinusoidal spaces between hepatic cords, degranulation and vacuolation of cytoplasm (Sharma *et al*., 2013).

MATERIALS AND METHODS

The 30 mice, male from strain of Blab/C (6-8 weeks of age, weighted 25-35 g) mice were obtained from the National Center For Drug Control and Research, Ministry of Health in Iraq, NCDCR they were kept for about 5 days before the onset of the experiment, under aseptic conditions. Animals were kept in metal cages with plastic base 15 cm, high and 25 cm with wood shavings at room temperature (20±5°C) and at normal daily 12 h, light/dark cycles. Commercial food pellets and tap water feed the mice. The mice were divided into 6 groups. The first group as the control group did not receive any radiation, second group was irradiated with 1711.152 μSv for 6 days and the third group was irradiated 3422.304 μSv for 12 days. The fourth group was irradiated 5133.456 μSv for 18 days fifth group was irradiated 6844.608 μSv for 24 days. While the sixth group irradiated 9126.144 μSv for 32 days.

Source of irradiation: Mice were irradiatedusing the Cesium source ($Cs^{\wedge 137}$) serial no. OX-840-Germany it was placed at the top of the cage 15 cm high and the activity 100 μCi. The block of lead has been placed in dimensions of (20×10 cm) around the cage to prevent radiation leakage into the laboratory.

Histopathological examination

Samples: Animals were sacrificed at the end of the experiment after inhalation anesthesia (chloroform) (Constan *et al*., 2002). Tissue samples or specimens of spleen and liver were taken from irradiated mice as well as control mice of the spleen were fixed in 10% formalin saline, trimming was done on the fixed tissue specimens and washed in tap water for 12 h. Serial alcohols (methyl, ethyl and absolute) were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 3μ thickness by slide microtome, the sectioning tissue slices were collected on the glass slides and stained with hematoxylin and eosin stain for histological examination by the light microscope (Wick *et al*., 2008).

RESULTS AND DISCUSSION

At the end of each period of exposure to gamma ray, animal's tissues then examined under the light microscope

Spleen specimens: At the early period 4 days of exposure to gamma ray splenic tissues showed marked depletion of white pulp in comparison to control an irradiated mouse as showed in Fig. 1a, b.

Splenic tissues after 8 and 12 days of exposure to gama ray showed depletion of white pulp with infiltration of mononuclear cells as megakaryocyte as appear in Fig. 2a, b.

Splenic tissues after 16 and 21 days showed depletion of lymphoid follicles and congestion of central arterioles and destruction of splenic inter trabecular septa infiltration of mononuclear cells mainly as shown in Fig. 3a, b.

Splenic tissues exposed to gamma ray for 24 and 32 days, showed destructive changes and deposition of heavy proteins material amyloid like substances within intracellular spaces as shown in Fig. 4a, b.

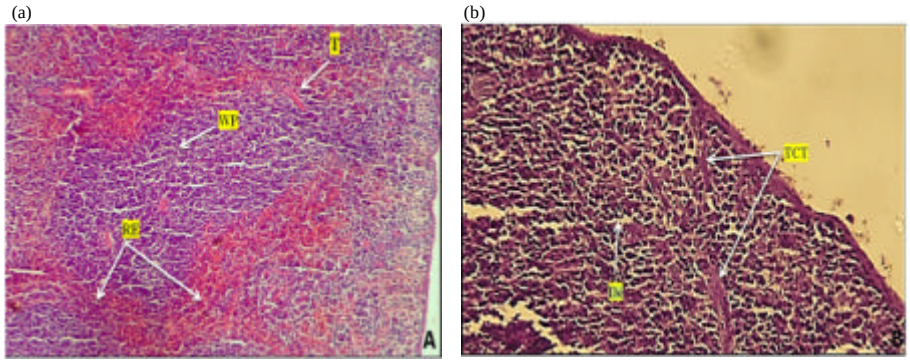

Fig. 1: a) Crros section spleen in control mice showing the normal structure of red, white pulp and Trabecular cord (H&E-X 200). (WP; Whit Pulp, RE; Red pulp, T; Trabecular cord) and b) Cross section of spleen of irradiated mice after 4 days, revealing depletion of white pulp (H&E-X200). (TCT; Connective Tissue Trabecular cord, De; Depletion of white pulp)

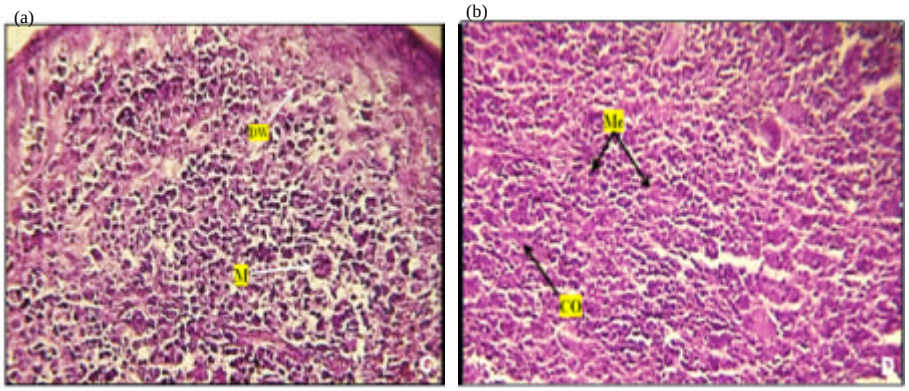

Fig. 2: a) Cross section of spleen of irradiated mice after 8 days, revealing depletion of whitepulp; mononuclear cells infiltration mainly megakaryocyte (H&E-X200). (M; Megakaryocytes, DW; depletion of white pulp) and b) Cross section of spleen of irradiated mice after 12 days, revealing the presence of megakaryocyte and increase in number (H&E-X200). (Me; Megakaryocyte, CO; Congestion)

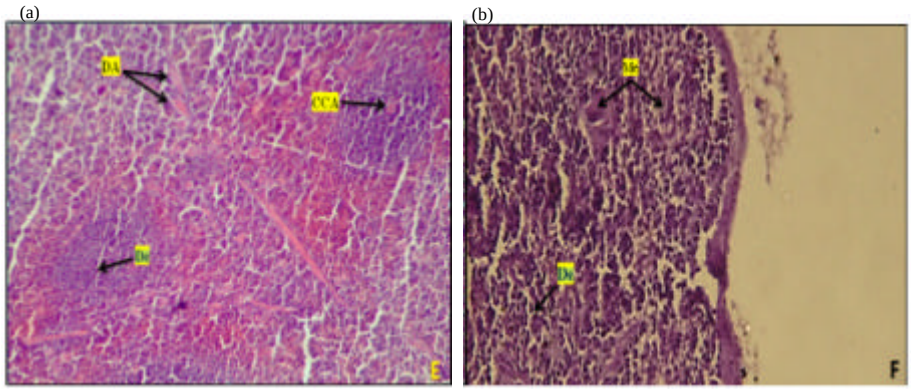

Fig. 3: a) Cross section of spleen of irradiated mice after 16 days, revealing depletion of lymphoid (H&E-X200). (De; Depletion of lymphoid follicles, CCA; Congestion in Central Arterioles, DA; Destruction of splenic trabecular septa) and b) Cross section of spleen of irradiated miceafter 21, section showing depletion and infiltration of mononuclear cells mainly megakaryocyte (H&E-X200). (Me; Megakaryocyte, De; Depletion of white pulp)

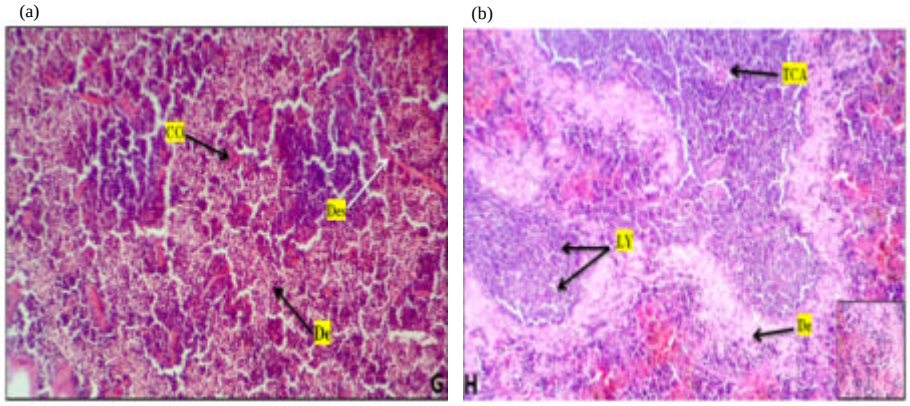

Fig. 4: a) Cross section of spleen of irradiated mice after 24, section showing severe destruction of spleen parenchyma with congestion of vessels an addition of starting for deposition of amyloid like substances (H&E-X200). (CO; Congestion of blood vessel, Des; Destruction splenic inter trabecular septa, De: Deposition of amyloid like substances and b) Cross section of spleen of irradiated miceafter 32 showing the deposition of amyloid like substances appear more clearly around leymphoied follicals (H&E-X200). (TCA; Thickened in Central Arterioles, ly; Lymphoied follicles, De; Deposition of amyloid)

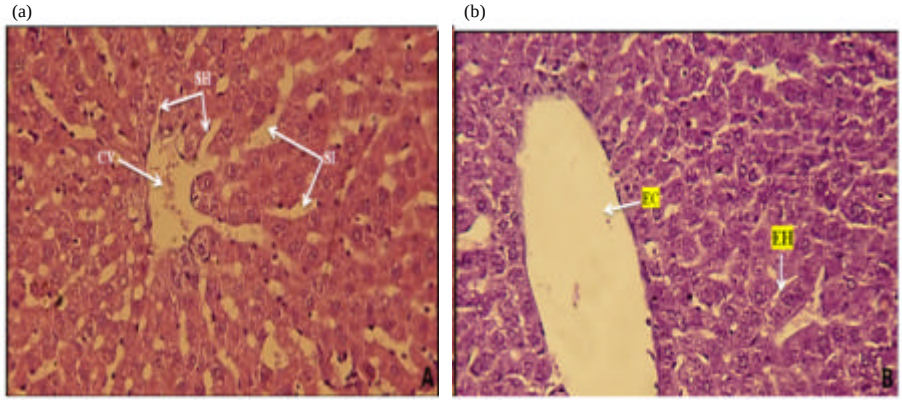

Fig. 5: a) Cross section of liver in control group showing the normal structure consist of central vein and sinusoids and sheet of hepatocytes (H&E-X200). (CV; Central Vein, Si; Sinusoids, SH; Sheet of Hepatocytes) and b) Cross section of liver of irradiated mice after 4 days, revealing enlargement of hepatocytes increase and absence of sinusoids and dilation of centralvein (H&E-X200). (EH; Enlargement of Hepatocytes, EC; dilation of central vein)

Liver samples: At early time 4 days of exposure to gama ray, liver of mice showed degenerative changes as swelling of hepatocytes which appear much enlarged with absence of sinusoids and dilation of central vein as compared to normal appearances of control group as showed in Fig. 5a, b.

Liver tissues of mice exposed to gama ray after 8 and 12 days showed infiltration of inflammatory cells, mainly kuffer cells; Expansion of central vein and complete loss of sinusoids due to swelling or enlargement of hepatocytes as shown in Fig. 6a, b.

Liver tissues of mice exposed to gama ray after 16 and 21 days showed huge enlargement of hepatocytes and congested blood within the dilated central vein; Necrotic area surround by inflammatory zone nearly to normally tissue's area as shown in Fig. 7a, b.

Liver tissues of mice exposed to gamma ray for 24 and 32 days showed focal aggregations of inflammatory cells which lead to formation of as shown in Fig. 8a, b.

Sections of the control group showed no abnormal lesions, spleen tissue showed to be consisted of white, red pulp and macrophage, the most characteristic lesion characterized by a depletion of white pulp appeared after 4 days post radiation while at 8 days post radiations, spleen showed depletion appeared more clearly in the presence of megakaryocyte this observation was in agree

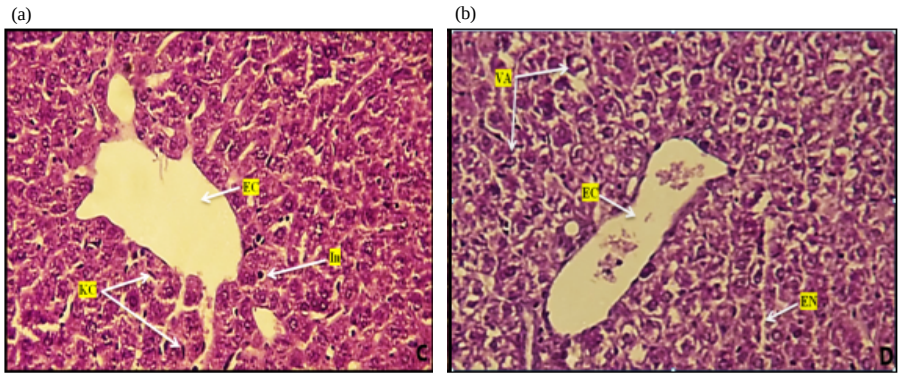

Fig. 6: a) Cross section of liver of irradiated mice after 8 days, revealing increase in the number of kuffer cells with infiltration with inflammatory cells in sinusoid at (H&E-X200). (EC; Extension of Central vein, KC; Kuffer cell, In; Inflammatory cells) and b) Cross section of liver of irradiated mice after 12 days revealing vacuolation and enlargement of hepatocytes and expansion of central vein with complete absence and sinusoid (H&E-X200). (EN; Enlargement of hepatocytes, VA; Vacoulation of hepatocytes, EC; Extension of Central vein)

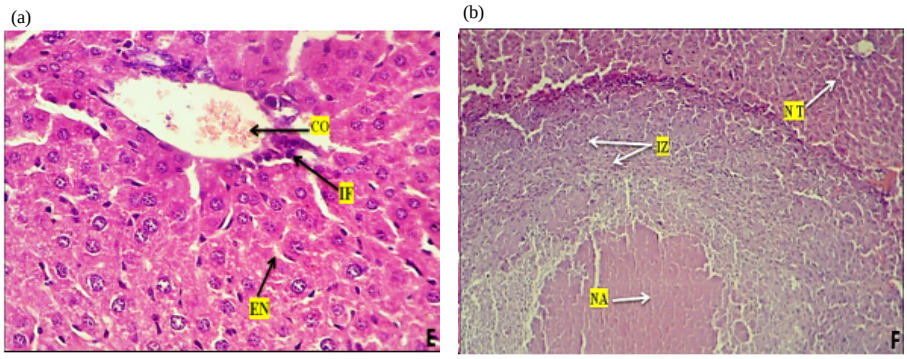

Fig. 7: a) Cross section of liver of irradiated miceafter 16 days, revealing the enlargement at hepatocytes appear more clearly with cuffing of inflammatory cells for central vein (H&E-X200). (EN; Enlargement of hepatocytes, CO; Congestion of central vein, IF; Inflammatory cells) and b) Cross section of liver of irradiated mice after 21 days showing large necrotic area appear surrounded by inflammatory zone which separated necrotic area of normal liver tissue (H&E-X200). (NA; Necrotic Area, IZ; Inflammatory Zone, NT; Normal Tissue)

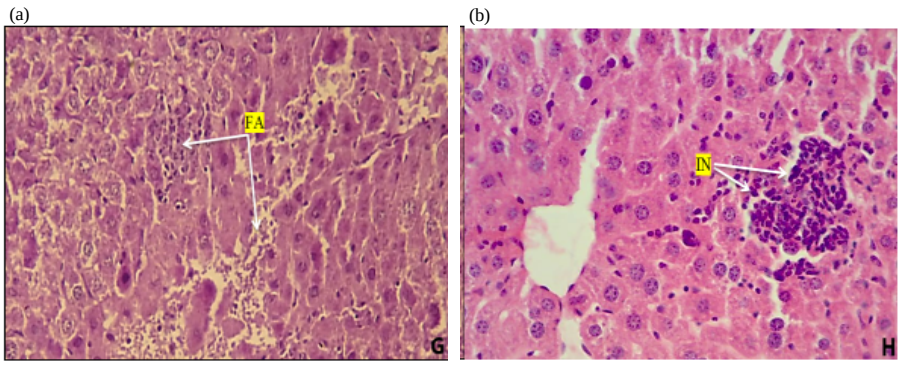

Fig. 8: a) Cross section of liver of irradiated mice after 24 days showing focal aggregation of infiltration cells appear with infiltration of inflammatory cells (H&E-X200). (FA; Focal Aggregation) and b) Cross section of liver of irradiated mice after 32 days, showing focal aggregation of infiltration cells appear with infiltration of inflammatory cells (H&E-X200). (IN; Infiltration of inflammatory cell giving formation of evidence of granuloma)

with (Abdou and Osman, 2008). Who noticed in a section of the spleen of gamma-irradiated rats marked dilation and congestion of splenic blood vessels as well as the presence of multiple megakaryocytic, studies of exposed animals and human indicate that radiation from cesium can also, hit a wide variety of tissues with higher levels of cellular divisions Blatt *et al*. (1994). The current study observed after 12 days radiation, the presence of a megakaryocyte which increased in number with severe depletion of white pulp in addition to infiltration of inflammatory cells this was agree with Sharma and Purohit (2012). Who documented that hematopoietic organs like spleen, thymus and bone marrow are markedly sensitive to the ionizing radiation and the clinical symptoms which are largely due to damage in the radiosensitive hematopoietic organs a very small dose of radiations of a blood forming organ causes an arrest of the hematopoiesis with changes in the peripheral blood at 16 days post radiation, spleen showed depletion of lymphoid while at 21 days the depletion appears more clearly in the presence of megakaryocyte and infiltration of inflammatory cells. Rahardjo *et al.* found that sinusoid area in the liver was increased in irradiated mice and the hepatic sinusoidal area irradiated increased in day 24 post inoculation compared to 6 days post radiation, the sinusoid area was wider in all days post radiation compared no radiated control group, the present study observed after 24 days post radiation, severe destruction of spleen parenchyma with congestion of vessels an addition of starting for deposition of amyloid like substances deposition of amyloid like substances appears more clearly around lymphoidfollicles after 32 days irradiation the histopathological changes in control appeared there was no lesion in all sections, liver consists of the central vein, sinusoids and the sheet of hepatocytes, glomerulus, vascular pole, tubules and capsular space was clearly observed in the most lesion of the liver characterized by enlargement of hepatocytes due to cell injury leading to absence of sinusoids at 4 days of radiation as shown in while after 8 days, showed increases in the number of kuffer cells with infiltration of inflammatory cells in a sinusoid. Moawad *et al.* (2016) determined that activation of kupffer cells may act both as effector cells in the destruction of hepatocytes by producing harmful soluble mediators and as antigen presenting cells during liver infection in liver damage and hepatocellular necrosis, activated kuffer cells are a major cause of inflammatory mediators (Kolios *et al*., 2006). At 12 days post radiation, vaculation and enlargement of hepatocytes with the complete absence of sinusoid were noticed while in 16 days, the enlargement at hepatocytes appear clearer with cuffing of inflammatory cells for central vein, many investigations showed that exposure to radiation increases free radical activity, the production of free radicals is considered to be the main cause of damaging effects, radiation induced lipid peroxidation decrease protein synthesis and proceeds disturbances in the enzyme activity of the liver (Kadiska *et al*., 2000). At 21 days after radiation, large necrotic area appears surrounded by the inflammatory zone which separated necrotic area from normal liver tissue, the lesion of liver progress at 24 days post radiation represented by the focal aggregation of infiltration cells appear with infiltration of inflammatory cells in many section at 32 days, the focal aggregation of infiltration cells appears with infiltration of inflammatory cells in many sections, more severe (giving formation of evidence of granuloma than 24 days. Roushdy *et al*. (1989) and El-Kafif *et al*. (2003) suggested that the reduction in liver function in irradiated rats might be the effect of either damage of biological membranes or to changes in the permeability of the liver.

CONCLUSION

The histopathological results showed distinctive patterns of spleen injuries in the irradiated group with liver necrosis and granulomatous lesion formation.

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REFERENCES

Abdou, M.I. and H.F. Osman, 2008. The modulation effect of melatonin against gamma irradiation in biochemical and histopathological studies on male rats. Isotope Radiat. Res., 40: 147-160.

Abojassim, A.A., H.S. Jaffat and A.B. Hassan, 2015. Effects of gamma radiation on some hematological parameters in female rats. ISJ Theor. Applied Sci., 5: 101-109.

Bala, M. and P. Kaur, 2015. Changes in spleen cell populations in total body 60 CO-gamma irradiated mice and their modification by SBL-1: Implication in radiation protection. Intl. J. Pharm. Sci. Rev. Res., 34: 109-113.

Blatt, D.R., W.A. Friedman, F.J. Bova, D.P. Theele and J.P. Mickle, 1994. Temporal characteristics of radiosurgical lesions in an animal model. J. Neurosurg., 80: 1046-1055.

Cesta, M.F., 2006. Normal structure, function and histology of the spleen. Toxicol. Pathol., 34: 455-465.

Constan, A.A., B.A. Wong, J.I. Everitt and B.E. Butterworth, 2002. Chloroform inhalation exposure conditions necessary to initiate liver toxicity in female B6C3F1 mice. Toxicol. Sci., 66: 201-208.

Deas, S.D., N. Huprikar and A. Skabelund, 2017. Radiation exposure and lung disease in today's nuclear world. Curr. Opin. Pulm. Med., 23: 167-172.

Dobrzynska, M.M., K.A. Pachocki, A. Gajowik, J. Radzikowska and A. Sackiewicz, 2014. The effect occupational exposure to ionizing radiation on the DNA damage in peripheral blood leukocytes of nuclear medicine personnel. J. Occup. Health, 56: 379-386.

Duncan, A.W., 2013. Aneuploidy, polyploidy and ploidy reversal in the liver. Semin. Dev. Biol., 24: 347-356.

El-Khafif, M., M. Ragab, H. El-Dawy and S. Tawfik, 2003. Effect of taurine treatment on some biochemical parameters in gamma irradiated mice. Environ. Sci., 6: 393-401.

Ezz, M.K., 2011. The ameliorative effect of Echinacea purpurea against gamma radiation induced oxidative stress and immune responses in male rats. Aust. J. Basic Appl. Sci., 5: 506-512.

Golla, S., J.P. Golla, K.W. Krausz, S.K. Manna and C. Simillion *et al*., 2017. Metabolomic analysis of mice exposed to gamma radiation reveals a systemic understanding of Total-body exposure. Radiat. Res., 187: 612-629.

Kadiska, M.B., B.C. Gladen, D.D. Baird, A.E. Dikalov and R.S. Sohal *et al*., 2000. Biomarkers of oxidative stress study: Are plasma antioxidants markers of CCl4 poisoning?. J. Free Rad. Biol. Med., 28: 838-845.

Kolios, G., V. Valatas and E. Kouroumalis, 2006. Role of kupffer cells in the pathogenesis of liver disease. World J. Gastroenterol., 12: 7413-7420.

Maharwal, J., R.M. Samarth and M.R. Saini, 2005. Antioxidative effect of Rajgira leaf extract in liver of Swiss albino mice after exposure to different doses of gamma radiation. Phytother. Res., 19: 717-720.

Moawad, M.A., M.M. Amin and E.N. Hafez, 2016. Hepatic histopathological and histochemical changes in mice infected with schistosoma mansoni after vaccination with gamma Radiation-attenuated schistosomules. Arab J. Nucl. Sci. Appl., 49: 188-198.

Mohamed, E.T., A.A. Elkady, E.S. Kiki and S.M. El, 2016. Protective effect of Symplocos racemosa roxb against gamma radiation induced cardiotoxicity in male albino rats. Pak. J. Zool., 48: 1831-1837.

Periasamy, P., J.K. Tan, K.L. Griffiths and H.C. O'Neill, 2009. Splenic stromal niches support hematopoiesis of Dendritic-like cells from precursors in bone marrow and spleen. Exp. Hematol., 37: 1060-1071.

Roushdy, H.M., M. El-Hussaini and F. Saleh, 1989. Effect of Whole-body Gamma-irradiation and/or dietary protein defeciency on the levels of plasma Non-protein-nitrogen and amino acids in desert rodents and albino rats. Egypt J. Rad. Sci. Appl., 1: 156-166.

Shahid, S., M.N. Chaudhry, N. Mahmood and S. Sheikh, 2015. Impacts of terrestrial ionizing radiation on the hematopoietic system. Pol. J. Environ. Stud., 24: 1783-1794.

Sharma, J., R. Sharma and A. Mathur, 2013. Protection of mouse liver from gamma ray exposure: A review. J. Intl. Pharm. Biol. Sci., 4: 1011-1026.

Sharma, R. and R.K. Purohit, 2012. Protective role of liv.52 against radiation and cadmium induced haematological changes in the Swiss albino mice. Int. J. Life Sci. Bt. Pharm. Res., 1: 114-123.

Soliman, M.G., O.M. Ashry, M.A. Ahmed and Y.H.A. El-Naby, 2015. Improvement of hematopoietic and immunologic findings in sublethal gamma irradiated rats treated with bone marrow transplantation and wheat germ oil. J. Immune Based Ther. Vaccines Antimicrobials, 4: 9-18.

Sutherland, B.M., P.V. Bennett, O. Sidorkina and J. Laval, 2000. Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation. Proc. National Acad. Sci., 97: 103-108.

Tanchev, S., S. Georgieva, D. Hristova, L. Sotirov and T. Koynarski *et al*., 2015. Life duration of inbred and outbreed rabbits, irradiated with gamma rays. Bulg. J. Agric. Sci., 21: 404-408.

Wick, M.R.M.D., C.M.H.T. Nancy, A.S.C.P. QIHC and K.B.M.D. William, 2008. Tissue Procurement, Processing and Staining Techniques. In: Diagnostic Histochemistry, Wick, M.R. (Ed.). Cambridge University Press, Cambridge, UK., ISBN:978-0-521-87410-6, pp: 1-27.