

The Protective Effect of BM Transplantation on Liver Tissue by a Chemical Carcinogen or γ - Radiation in Rats.

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ABSTRACT

Aim of work: this work aimed to study the biochemical and histopathological changes in the liver of male albino rats post exposure to 6Gy of gamma radiation and the possible protective effect of bone marrow (BM) transplantation on the liver tissues by a chemical carcinogen ferric nitrilotriacetate (Fe-NTA) or γ - radiation in rats.

Materials and methods: in this study, thirty six healthy and active male albino rats about 120 grams in body weight were used. The animals were housed in plastic cages under normal temperature, pressure, humidity and good ventilation conditions during the whole period of the experiment. The animals were fed on a standard pellet diet and water. Animals were categorized into six groups and served as the following groups: control, gamma irradiated (R), Fe-NTA, BM+R, BM + Fe-NTA and BM.+Fe-NTA+R.

Results: the present results suggested that exposure to γ -radiation or Fe-NTA induced a significantly disturbance in the liver functions and structure. They increased significantly the oxidative stress and decreased significantly the antioxidants tissues and they also increased necrotic and apoptotic cells in rat's liver tissue.

Bone marrow transplantation either after whole body gamma-irradiation or Fe-NTA treatment restored the liver functions and structure. BM also ameliorated the oxidative stress and antioxidative markers.

The histopathological observations recorded some amelioration in the apoptotic and necrotic evaluation in liver tissue.

Keywords: γ - Radiation, ferric nitrilotriacetate, bone marrow, rats.

INTRODUCTION

Ionizing radiations cause similar damage at the cellular level. Gamma rays and neutrons are more penetrating, causing diffuse damage throughout the body (e.g. radiation sickness, cell's DNA damage, cell death due to damaged DNA, increasing incidence of cancer) rather than burns. The most biological damaging forms of gamma radiation occur in the gamma ray window, between 3 and 10 Megaelectron-volts (MeV) [1].

When male Wistar rats were exposed to a single dose of 6 Gy whole body gamma radiation, the results showed a highly significant increase in the levels of plasma AST and ALT during three weeks post-irradiation [2]. Exposure of rats to both doses (1 & 6Gy) of gamma radiation induced a significant increase in plasma AST and ALT levels as a dose dependent manner after 2 hours, 2 days till 2 weeks postirradiation. Similar results were reported by Ramadan *et al.* [3] after 1 and 6 days post irradiation at dose levels of 2 and 6 Gy in male rat. Ferric

nitrilotriacetate (Fe-NTA), a free radical generating compound, is formed by the interaction of iron and nitrilotriacetic acid [4]. It is a synthetic chelating agent used in various countries as a constituent in detergents and it is known to catalyze *in vitro* hydroxyl radical production from H₂O₂ more efficiently than does Fe(III) [5].

Fe-NTA has been shown to induce hepatic oxidative damage in HepG2 cells [6]. Fe-NTA induced hepatotoxicity in rats and increased values of both alanine transaminase and aspartate aminotransferase [7]. The prevention of Fe-NTA induced hepatotoxicity by vitamin E has also been reported by Agarwal *et al.* [8].

Vossen [9] and El-Ganzuri *et al.* [10] reported that autologous or syngeneic and allogeneic bone marrow transplantation (BMT) is increasingly used in the therapy of lympho-hematopoietic and solid malignancies, as well as in non-malignant disorders such as thalassemia and immunodeficiency. Also, the preclinical and clinical study demonstrated

that bone marrow stromal cells (MSCs) can be used for tissue repair^[11].

MATERIALS AND METHODS

Adult male Wister rats (average weight 150 gm) were purchased from the laboratory of Experimental Serum and Vaccine Authority. Animals were fed on a pellet diet and *ad libitum*.

Radiation treatment

Whole body γ -irradiation was performed with a Canadian 137Cs Gamma Cell-40 at the National Centre for Radiation Research and Technology, Cairo, Egypt. The rats were exposed to a single dose of 6Gy whole body γ - radiation at a dose rate of 1.2 rad/second.

Bone Marrow Transplantation:

Donors and recipients rats were chosen of the same inbred strain, brother to brother. The femur bones were dissected out and cleaned. Ends of the bones were chipped by a bone nibbling forceps. The bone marrow (BM) was blown out of the femur into isotonic solution under sterilized conditions inside a laminar flow cabinet. The bone marrow was collected into a sterile container surrounded by ice cubes and mixed by drawing and expelling it several times from the syringe without needle in order to avoid mechanical damage to the cells. Total viable cells of about $75 \times 10^6 \pm 5\%$ were injected intravenously (IV) through the caudal vein.

Groups of animals under investigation:

Thirty six animals divided into 6 groups, 6 rats each.

Group1: served as control group, animals were injected intraperitoneally by only saline solution at a single dose of 9 mg/kg b.w. (**Control**).

Group2: animals were intraperitoneally injected acarcinogen compound(Fe-NTA) twice a week at a dose level 9 mg/kg body weight (**Fe-NTA treated group**) (*Awai et al.*)^[12]

Group3: animals of this group were exposed to 6 Gy of whole body γ -radiation (**R**).

Group4: animals of this group were exposed to 6 Gy of γ -radiation and injected with BM cells three hours after γ -radiation exposure(**BM+R**).

Group 5: animals received a single dose of Fe-NTA at the same doses, followed by post-treatment with BM cells; the animals of this group were IV injected with BM cells ($75 \times$

106) cells through the caudal vein(**Fe-NTA+BM**).

G6: animals of this group were intraperitoneally injected with Fe-NTA twice a week, exposed to 6Gy of γ - rays and injected with bone marrow cells one month before killing the animals(**Fe-NTA+R+BM**).

Sample collection:

1-Blood sampling:

After the animals of the above groups were sacrificed, blood samples were collected by heart puncture and aspiration of the heart blood was done using plastic syringe. Another amount of the blood was collected in heparinized glass tube for the biochemical and chemical analyses.

2-Tissue sampling:

After the animals of the above groups were sacrificed, small pieces of liver tissue were quickly removed. Samples were put in 10 % neutral formalin for the histopathological study. Another samples of the tissues were perfused immediately with ice-cold saline (0.85% w/v NaCl) and homogenized (Glas-Col, Terre Hauter, USA) in chilled phosphate buffer (0.1 M, pH 7.4) which contains KCl (1.17% w/v). The homogenate was centrifuged at 3000 rpm for 10 min at 4°C in refrigerated centrifuge to separate the nuclear debris.

The histopathological study:

The sacrificed animals were quickly dissected. Sample of 0.5cm³ of the liver organ was removed and fixed in 10% neutral formalin for 24 hours followed by washing, dehydration in ascending grades of alcohol, clearing in xylene and they were embedded in hard paraffin. Samples were then serially sectioned at thickness of 5 μ m. Sections were mounted on albuminized slides and left for 24 hours at 37C to dry and to avoid detachment of sections during subsequent steps of staining. Sections were stained by hematoxylin and eosin stain **according to Harris**^[13] and then examined under the light microscope.

The biochemical study:

In the biochemical study nitric oxide was determined in plasma and in tissue homogenate according to the method of **Miranda et al.**^[14] and catalase activity as antioxidant markers according to the colorimetric method of **Sinha**^[15]

Assessment of aspartate and alanine transaminases (AST and ALT) activity.

The activity of serum aspartate and alanine transaminases (AST and ALT) in liver was measured according to the method of **Reitman and Frankel**^[16]

Statistical analysis:

Statistical analysis was carried out to compare between the different groups by one way ANOVA using SPSS (Statistical Package for Social Science, version 20) according to the method of **Daniel**^[17] and **Bailey**^[18]. The significance among the groups was compared at $P \leq 0.05$.

Immunohistochemical studies:

Apoptotic stain:

It was used to detect DNA damage associated with apoptosis by using acridine orange solution according to **Ribble *et al.***^[19].

RESULTS

Biochemical results:

Table 1- Represented the level of liver function tests in the different experimental animals groups.

Bone marrow transplantation recorded a non-significant change either for ALT or AST levels compared to the control level.

Exposure of rats to γ -radiation recorded a significant increase in liver function tests. The increase was 61.8% for ALT and 95.8% for AST compared to the control level. Also, the combined treatment of rats with Fe-NTA and γ -radiation represented great alterations in liver function tests and showed a significant increase in AST and ALT.

Treatment of rats with Fe- NTA and /or γ -radiation and bone marrow transplantation represented an amelioration in liver functions tests.

Treatment of rats with γ -radiation or/and Fe-NTA showed a significant increase in liver NO level with percent of change 37.1, 53.4 respectively ($P < 0.05$) compared to the control group (**Table 2**).

The present study showed that treatment of the experimental animals with Fe-NTA and /or γ -radiation groups with bone marrow transplantation showed a significant decrease in liver NO level compared to the untreated Fe-NTA, γ -radiation and Fe-NTA+ γ -radiation groups.

Treatment of rats with γ -radiation or Fe-NTA showed a significant decrease in catalase liver tissue with percent of change -58.9 and 65.5 respectively ($P < 0.05$) compared to the control group. Treatment of the experimental animals with Fe-NTA or γ -radiation with bone marrow transplantation showed a significant increase in liver catalase level compared to the Fe-NTA or γ -radiation untreated groups.

➤ Histopathological Observations

A- Hematoxylin and eosin (H&E) staining

(**Figure 1**) showed the normal architecture of hepatic tissue of a control rat. Treatment of rats with Fe- NTA showed congested blood vessel of the liver which contain hemolysed blood cells, lymphocytic infiltration, debris of degenerated cytoplasmic organoids, pyknotic and karyolytic nuclei in hepatocytes with increased apoptotic and necrotic hepatocytes (**Figure 2**).

Exposure of the animals to 6Gy γ -radiation induced pathological signs in the liver cells such as vacuolization, cytoplasmic degeneration and pyknotic nuclei of hepatocytes. Necrosis increased in liver cells, ruptured walls of the central veins with presence of hemorrhagic areas in the liver tissue were also detected (**Figure 3**). However, treatment of rats with 6Gy γ -rays rats with bone marrow transplantation induced some amelioration and recovery in the hepatic cells and the central veins (**Figure 4**). On the other hand, liver sections of rats treated with Fe-NTA and BMC transplantation represented disappearance of ruptured walls of the central veins, but some vacuolated hepatocytes with some hemorrhagic and necrotic areas were still detected (**Figure 5**).

On the other hand, liver tissue of rats treated with Fe- NTA and exposed to γ -radiation with BMC transplantation showed highly dilated central veins contained some haemolysed blood cells, congested hepatic portal veins with inflammatory cellular infiltrations (**Figure 6**).

DISCUSSION

Humans on earth are exposed to many sources of ionizing radiation. The largest component of man-made background radiation relates to exposures associated with medical diagnosis and treatments. **Malik *et al.***^[20] revealed that radiation therapy can produce significant tissue injury. On the other hand, bone marrow

cells suppress immune cell responses and have beneficial effects in various inflammatory-related immune disorders^[21].

On the other hand, epidemiological study of **Smith et al.**^[22] have shown that increased body iron storage is associated with an increased risk of cancer and early death. An experimental study had demonstrated that iron overload dramatically potentiates chemical carcinogenicity mechanisms whereby iron may act in carcinogenesis are induction of oxidative stress, facilitation of tumor growth and modification of the immune system. Metal ions react with superoxide anion ($O_2^{\cdot-}$) and H_2O_2 to produce highly reactive species such as hydroxyl free radical ($\cdot OH$) and metal-oxygen complexes in biological systems, resulting in oxidative DNA damage. Since H_2O_2 itself is not toxic to cells, H_2O_2 -induced oxidative DNA damage in cells has been thought to result from the formation of hydroxyl free radicals through Fenton reaction with iron^[23]. Nitriilotriacetic acid (NTA) is an aminotricarboxylic acid with an empirical formula of $C_6H_9NO_6$. Nitriilotriacetate (NTA) can make complexes with metal ions such as Fe^{3+} or Cu^{2+} . Fe-NTA, a complex of Fe^{3+} and NTA, is a strong nephrotoxic agent and a renal carcinogen. It is an established fact that an iron-chelate of nitriilotriacetate, ferric nitriilotriacetate (Fe-NTA) induces acute and sub-acute injuries in animals^[12,24,25]. It is evidenced from previous reports that oxygen free radical was formed from redox-active iron and was detected in the serum of Fe-NTA-treated rats^[27].

Therapeutic studies for bone marrow transplantation for treatment of radiation injuries and its consequences are not more available. Thus, this work investigated the possible protective role of the bone marrow cells against damage induced in rat's organs by chemical carcinogen Ferric nitriilotriacetate (Fe-NTA) and γ -radiation exposure. The present investigations were performed to evaluate the changes in liver functions and also to evaluate the change of nitric oxide, catalase levels. Histopathological changes in liver tissue, apoptotic and necrotic investigations were also evaluated in liver tissue.

In the present study, whole body exposure of rats to 6 Gy induced alteration in liver functions, obvious by significant increases in the activity of serum AST and ALT compared

to control values. The significant increase in the activity of serum ALT and AST might result from radiation-induced liver cell membrane damage and alterations in dynamic permeability of membranes due to peroxidation, which is followed by the release of intracellular enzymes to the blood stream.

The present results also showed marked increase in plasma ALT and AST activities of Fe-NTA treated rats compared to the control group and this agreed with previous study of **Kaur et al.**^[28]. Others demonstrated that the level of the liver enzymes increased in plasma of Fe-NTA injected rats indicating general toxicity that occurred due to hepatic injury thus serving a main organ for maintaining homeostatic conditions^[29].

Also **Lee and Levine**^[30] mentioned that, the most common complications in cancer patients are malnutrition, gastrointestinal disturbance and liver dysfunction. The most sensitive markers employed in the diagnosis of hepatic dysfunction are ALT and AST because they are located in the cytoplasm of hepatocytes and are released into the circulation after cellular damage^[32].

Our results show that whole body γ -irradiation of rats at 6Gy showed a significant increase in rat's liver tissue of nitric oxide (NO) levels. Similar results have been reported by **Mansour et al.**^[33] γ -irradiation may enhance endogenous NO biosynthesis in liver, intestine, kidney, lung, brain, spleen or heart of the animals, presumably by facilitating the entry of Ca^{2+} ions into the membrane as well as the cytosol of NO-producing cells, though irradiation-induced membrane lesions. The enhancement of NO production following exposure to a high dose (6 Gy) of g-rays was attributed to high levels of expression of the inducible nitric oxide synthase^[34]. NO have been suggested to be involved in acute radiation response in tissues such as the liver, intestine, colon, and brain^[35]. Nitric oxide plays an important role in inflammation and carcinogenesis and has now been implicated as an important signaling molecule under normal physiological conditions. Increased NO results in increased nitration of proteins at tyrosine, which can cause protein dysfunction or alterations in signal transduction pathways^[36]. On the other hand, ionizing radiation has been confirmed to potentiate NO production in macrophages. The increase of

NO production in irradiated macrophages contributed to tumoricidal activity, with the activation mechanisms differing between high-dose and low dose irradiation^[34].

Also **Ali *et al.***^[37] showed that Treatment with Fe-NTA caused significant reduction in glutathione level, glutathione peroxidase, catalase and superoxide dismutase activities with marked elevation in lipid peroxidation, nitric oxide.

There is an increasing evidence that cancer and other mutation related diseases can be prevented not only by avoiding exposures to recognized risk factors but also by favoring the intake of protective factors and by modification of defense and DNA repair mechanisms of the host organism^[37].

Bone marrow transplantation (BMT) is the treatment of choice for many leukemias, solid tumors, and metabolic diseases^[38].

In the present study treatment of Fe-NTA and /or γ -irradiation groups with bone marrow transplantation represented amelioration in liver functions tests.

The process of BMT should be termed hematopoietic cell transplantation or hematopoietic stem cell transplantation, because the stem cells responsible for reconstituting the immune system can now be harvested directly from the circulation. Currently, most transplants deliver peripheral-blood-mobilized stem cells and not cells harvested directly from the BM by aspiration.

In the present study treatment of experimental animals with Fe-NTA and /or γ -irradiation groups with bone marrow transplantation showed a significant decrease in nitric oxide levels in liver tissue compared to the untreated groups this is agree with **Maha *et al.***^[39] .who found that In irradiated animals receiving BMT significantly depressed in Lipid peroxidation in serum and tissue were conversely elevated as compared with the irradiated group.

On the other hand treatment with Fe-NTA and /or γ -irradiation groups with bone marrow transplantation showed a significant increase in Liver tissue, in glutathione , glutathione peroxidase and catalase levels compared to the Fe-NTA and /or γ -irradiation untreated group.

Histopathological Observations:

Ionizing radiation causes a number of various cytological, histological injuries^[40]. There is a highly radiosensitive tissue (bone marrow

and lymphoid tissue) and moderately radiosensitive in liver tissue.

Context with the finding of **Jaya *et al.***^[41] histopathological observations in liver tissues of the experimental animals exposed to γ -radiation show vacuolization, degenerated cytoplasm and pyknotic nuclei of the hepatocytes. Hepatic necrosis and rupture of the central vein with presence of hemorrhage were also observed in the liver tissue. Also, Propidium iodide and acridine orange (PI/AO) show some necrotic cells and appearance of apoptotic hepatocytes. Cohen (2002) explained that tissue injury caused by ionizing radiation is initiated by oxidative injury to deoxyribonucleic acid. It is established that tissue injury elicits acute inflammation whose features among others include swelling of the affected part.

Also, **Mona and Mervat**^[42] study the therapeutic effect of bone marrow transplantation for treatment of radiation injuries. Surprisingly treatment of irradiated rats with bone marrow decreased the harmful effects of radiation. In addition to improving the expression of the studied genes to near the control levels, these models directed hepatic cells toward cell survival as noticed by the decreased Bax/bcl-2 ratio. On the other hand bone marrow is the major reservoir for adult organ-specific stem cells, including endothelial progenitor cells, haematopoietic stem cells, and mesenchymal stem cells^[43]. consequently, it is reasonable to propose that BM mesenchymal stem cells may be locally activated in the livers of the irradiated rats and compensated the damaged cells. Bone marrow contains hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC), which may derive from a common primitive blast like cell precursor able to differentiate along MSC or HSC potentials^[44].

Conclusions-Bone marrow transplantation either after whole body gamma radiation or Fe-NTA treatment restored the liver and kidney functions

and structure. It also ameliorated the oxidative stress and antioxidants markers.

The histopathological observations recorded some amelioration in the structure, apoptotic and necrotic evaluation in liver tissues. So, BM transplantation exerts some curative effect on the function and histological structure of liver of rats exposed to gamma-irradiation or Fe-NTA treatment.

From the previous results we can conclude that BM transplantation could protect liver of rats against radiation or Fe-NTA induced changes .

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Table1 -Effect of bone marrow transplantation on liver functions tests of Fe-NTA and /or radiation treated rats

Parameters Groups	ALT (U/L)	AST (U/L)
G1: Control Mean±SE % of change from G1	40.1±0.5 0	42.7±0.4 0
G2: Fe-NTA Mean±SE % of change from G1	83.7±1.3 ^{abc} 108.7%	96.7±0.8 ^{abc} 126.5%
G3: R. Mean±SE % of change from G1	64.9±0.6 ^{ac} 61.8%	83.6±0.9 ^{ac} 95.8%
G4:BM + R Mean±SE % of change from G1	77.3±1.2 ^{ab} 92.8%	91.3±0.9 ^{ab} 113.8%
G5: BM + Fe-NTA Mean±SE % of change from G1	49.7±1.2 ^{abc} 23.9%	59.3±1.8 ^{abc} 38.9%
G6:BM+R+ Fe-NTA Mean±SE % of change from G1	45.4±1.1 ^{bc} 13.2%	44.6±1.2 ^{bc} 4.4%

All values are expressed of 10 animals as Means ± standard Error (M±SE).

a: significant against G1 control group.

b: significant against G3 radiation group.

c: significant against G5 Fe-NTA group.

All significance at P ≤ 0.05.

G1: control animals. G2:BM group. G3: R group. G4: R+BM group. G5: Fe-NTA group. G6: R+ Fe-NTA group. G7: Fe-NTA+BM group. G8: Fe-NTA+R+BM group.

Table 2- Effect of bone marrow transplantation on nitric oxide levels of blood and tissue organs of Fe-NTA and /or γ-radiation treated rats

Parameters Groups	NO (nM/g wet tissue)	Catalase (µmol/g wet tissue)
G1: Control Mean±SE of change from G1	59±2.6 0	99.7±1.5 0
G2: Fe-NTA Mean±SE % of change from G1	109.7±2.3 85.9%	27.8±1.1 -72.1%
G3: R. Mean±SE % of change from G1	80.9±1.2 37.1%	40.9±1.1 -58.9%
G4:BM + R Mean±SE % of change from G1	90.5±2.1 53.4%	34.4±3.6 -65.5%
G5: BM + Fe-NTA Mean±SE % of change from G1	80.6±1.8 36.6%	80.3±2.2 -19.5%
G6:BM+R+ Fe-NTA Mean±SE % of change from G1	60.3±1.5 2.2%	95.7±1.1 -4.0%

Legends as in Table (1)

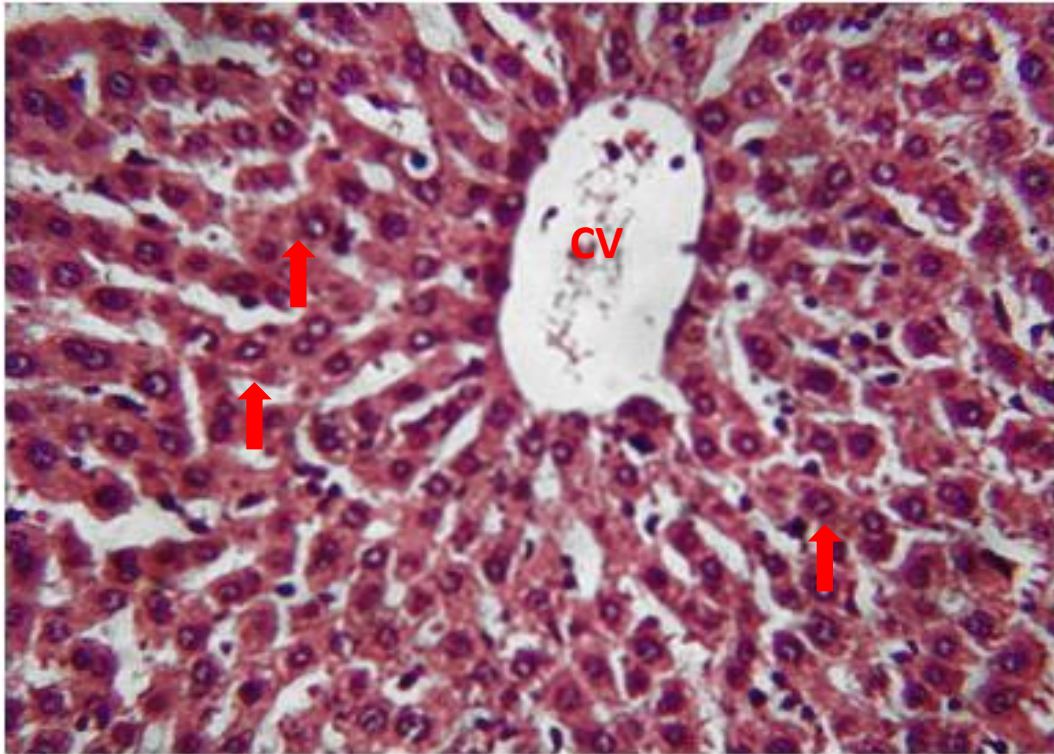


Figure 1: Light micrograph of liver tissue section of control rat shows the normal lobular pattern of the liver with a centrilobular vein (CV), hepatocytes with their nuclei (↑) and intervening blood sinusoids in between them. (H &E X 400)

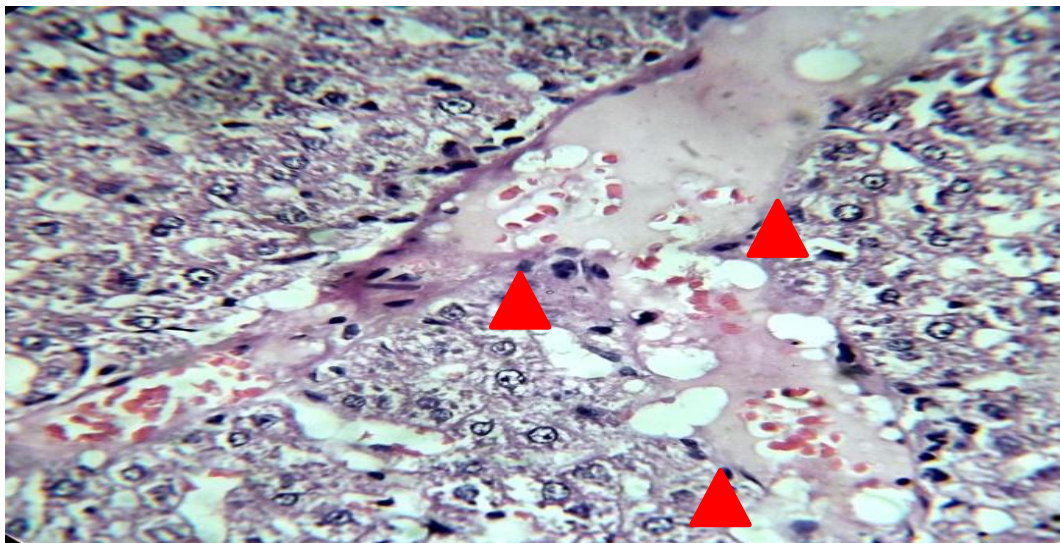


Figure 2: Light micrographs of liver tissue of a rat treated with Fe-NTA showing congested blood vessel (▲) contains haemolysed blood cells and lymphocytic infiltration, debris of degenerated cytoplasmic organelles with pyknotic and karyolytic nuclei. (H &E X 400)

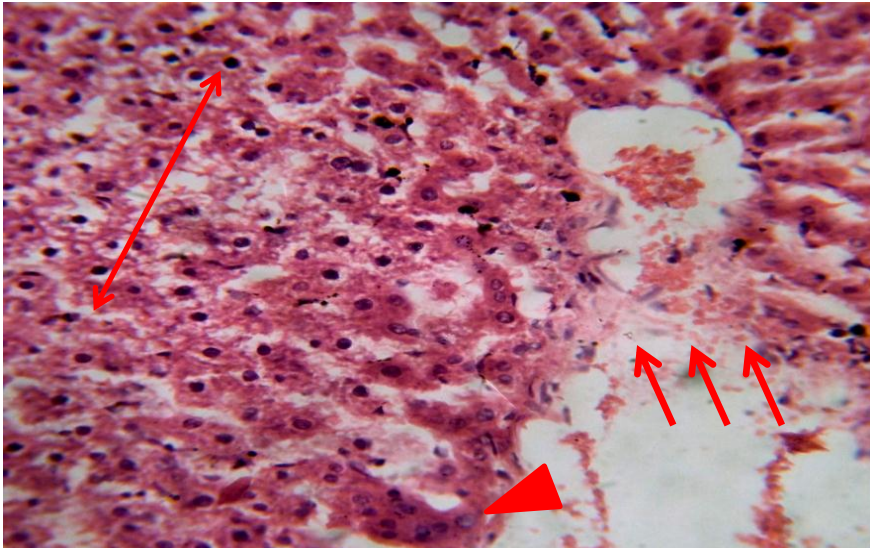


Figure 3:Light micrographs of liver sections in a rat exposed to 6Gy γ -radiation. Liver sections show gradual vacuolization, cytoplasmic disintegration and pyknotic nuclei (\Downarrow). Hepatic Necrosis (\blacktriangleleft) and great ruptured wall of the central vein with the presence of haemorrhage were also observed ($\Uparrow\Uparrow\Uparrow$). (H & E X 400).

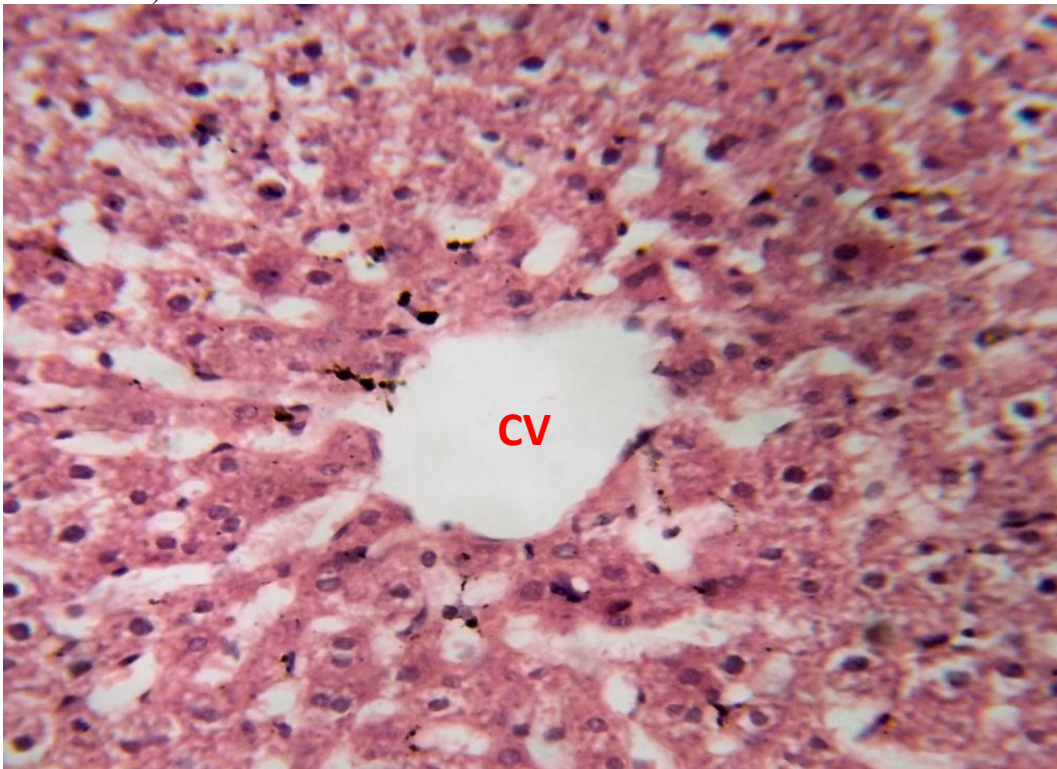


Figure 4- Light micrograph of liver tissue of a rat treated with 6Gy γ -radiation and BMC transplantation. Liver section shows signs of recovery in the central vein branch (CV) and hepatic cells. (H & E X 400).

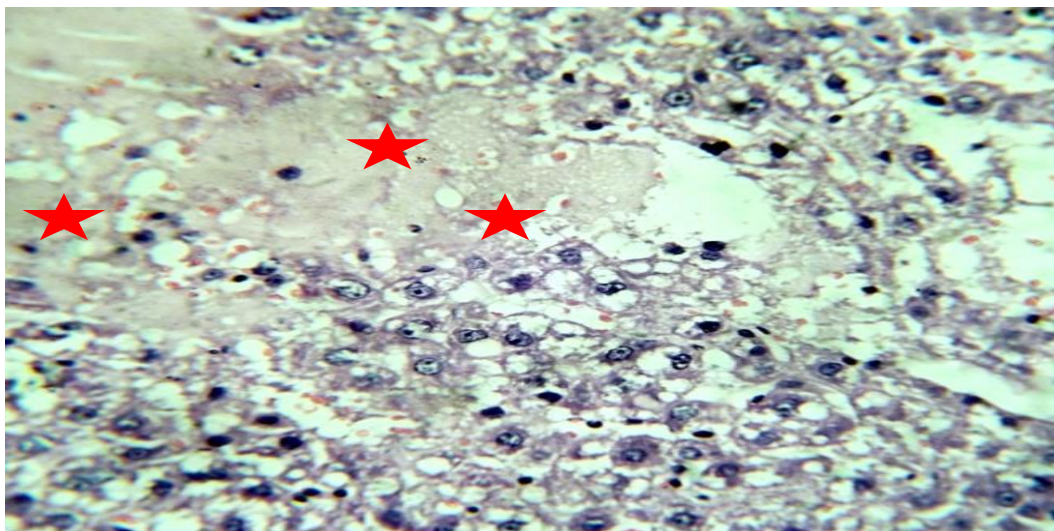


Figure 5-Light micrograph of liver tissue of a rat treated with Fe- NTA and BM transplantations showing hemorrhagic () and degenerated areas (H &E X 400)

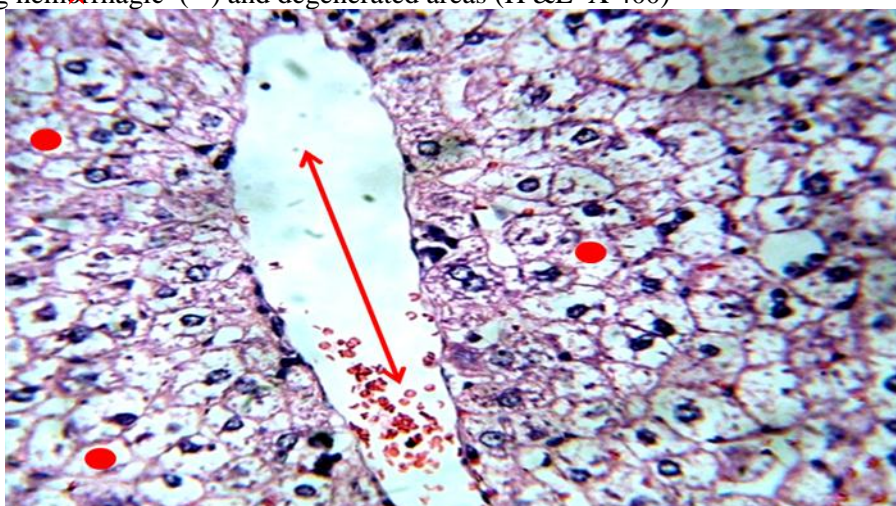


Figure 6- Light micrograph of liver tissue of a rat treated with Fe- NTA, exposed to γ -radiation and BMC transplantation showing highly dilated central vein (↓) which contains some hemolysed blood cells. Most hepatocytes are vacuolated with pyknotic or karyolytic nuclei (●). (H&E X400)

B- Propidium iodide and acridine orange (PI/AO) stain

Apoptotic and necrotic cells stained by PI/AO stain and examined under a fluorescent microscope. Compartments of the liver tissue in normal rat Fluorescent microscopic picture of control liver of male rat showing the major of viable cells in liver tissue. (Figure 7). Treatment of rats with Fe- NTA showing the highly appearance of necrotic cells and apoptotic hepatocytes (Figure 8) in the liver tissue.

Liver sections in a rat exposed to 6Gy γ -radiation show some necrotic cells, the appearance of apoptotic hepatocytes and empty places (Figure 9)

On the other hand, Liver sections of a rat exposed to 6Gy γ -radiation and treated with BMC transplantation show the normal appearance of liver tissue section and few apoptotic hepatocytes (Figure 10).

Meanwhile, fluorescent microscopic picture of liver section in a rat treated with Fe- NTA and BMC transplantation show the normal appearance of liver tissue section. (Figure 11).

However highly appearance of necrotic cells and apoptotic hepatocytes were detected in liver cortex of rats treated with Fe-NTA, exposed to γ -radiation and treated with BMC transplantation (Figure 12).



Figure 7: Fluorescent microscopic picture of control liver of male rat showing the major of viable cells in liver tissue. (PI/AO stain. B X250)

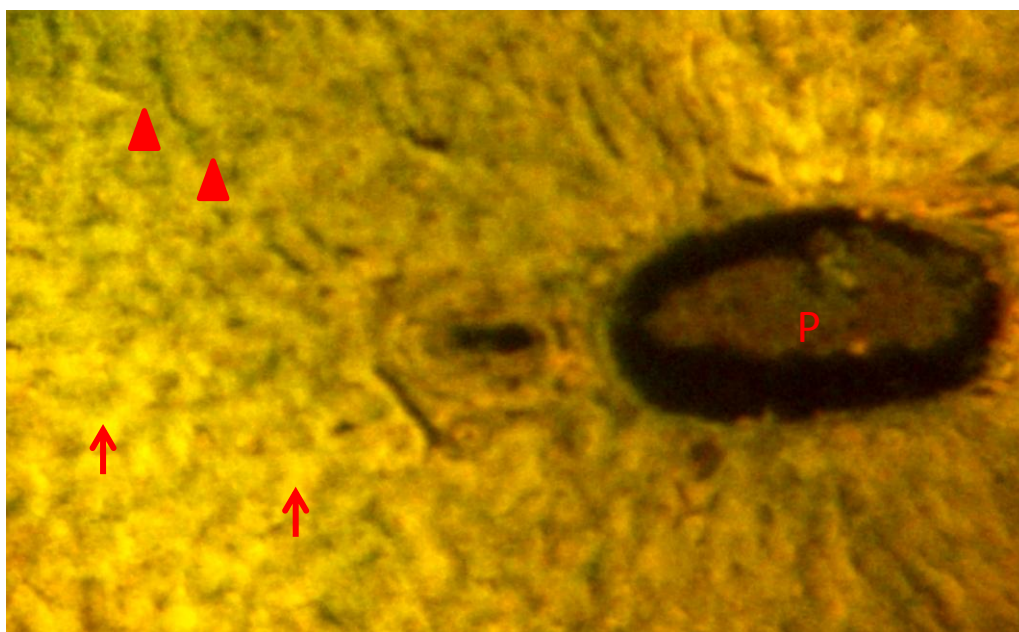


Figure 8: Fluorescent microscopic pictures of liver sections in a rat treated with Fe- NTA showing the highly appearance of necrotic (▲) and apoptotic hepatocytes (↑). (PI/AO stain. B X250)

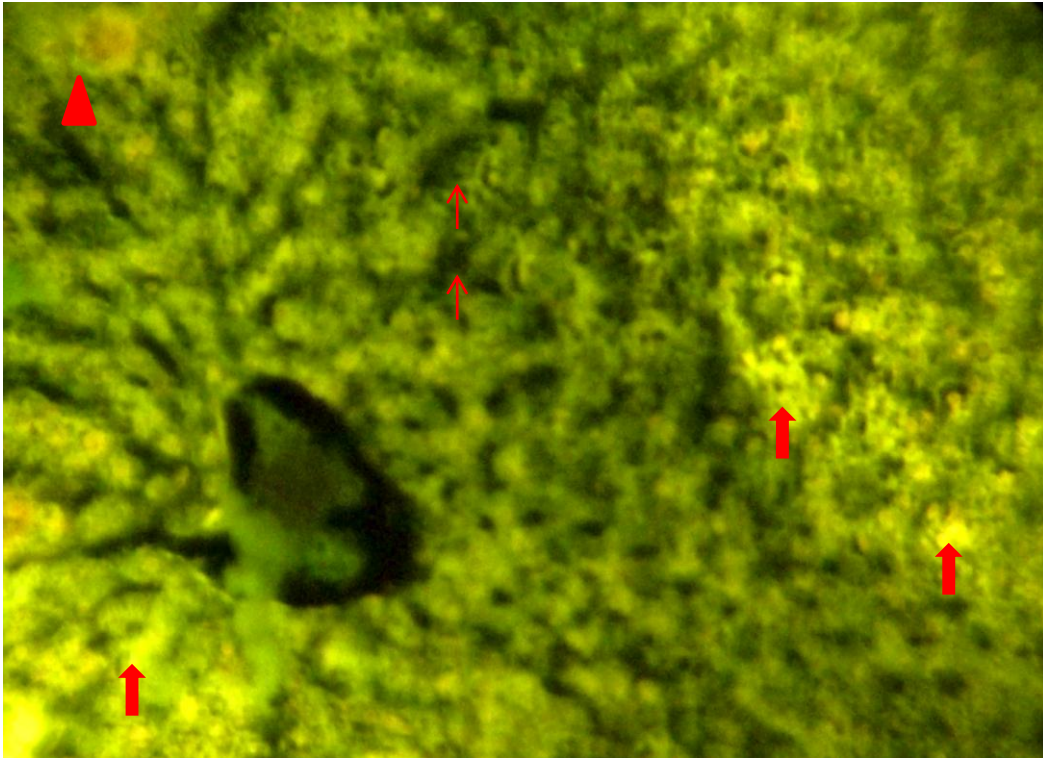


Figure 9: Fluorescent microscopic pictures of liver sections in a rat exposed to 6Gy γ -radiation show some necrotic cells (\blacktriangle), the appearance of apoptotic hepatocytes (red bold arrow) and empty places (\uparrow). (PI/AO stain. B X250)

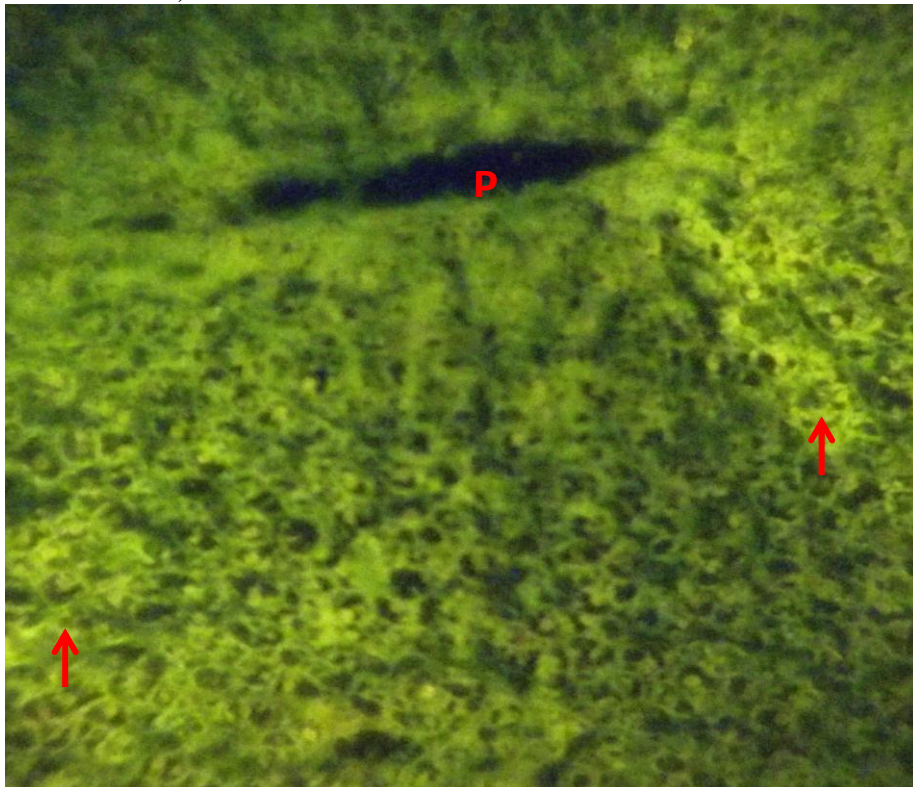


Figure 10: Fluorescent microscopic pictures of liver sections in a rat exposed to 6Gy γ -irradiation and treated with BMC transplantation show the normal appearance of liver tissue section and few apoptotic hepatocytes (\uparrow). (PI/AO stain. B X250)



Figure 11: Fluorescent microscopic picture of liver sections in a rat treated with Fe- NTA and BMc transplantation show the normal appearance of liver tissue section. (PI/AO stain. B X250)

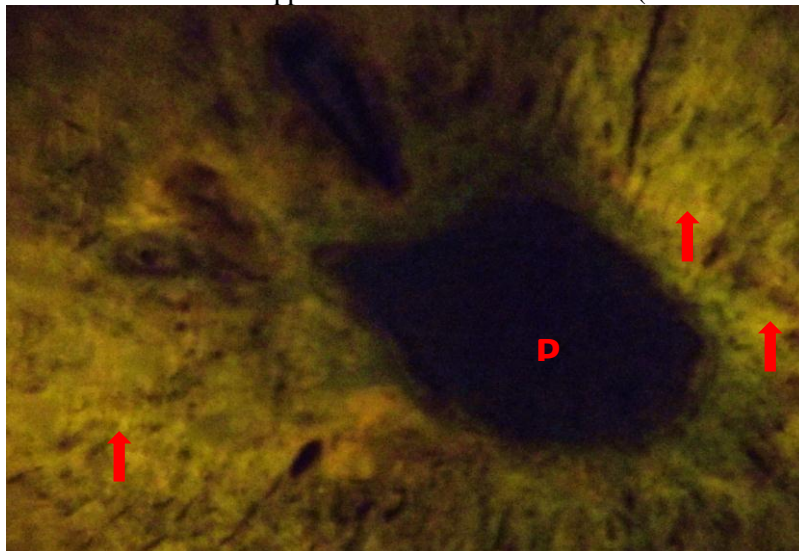


Figure 12: Fluorescent microscopic pictures liver tissue of a rat treated with Fe- NTA exposed to γ -radiation showing the highly appearance of necrotic hepatocytes (red bold arrows). (PI/AO stain. B X250)