The Protective Role of Transplanted Bone Marrow Cells against Injuries Induced by a Chemical Carcinogen and / or γ-Rays in Kidney tissue of Rats.

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ABSTRACT

Aim of the work:this work aimed to study the biochemical and histopathological changes in the kidney of male albino rats post exposure to 6Gy of gamma radiation and the protective role of transplanted bone marrow cells against damage induced in rat's kidney by a chemical carcinogen.

Materials and Methods: in this study, forty eight 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 experimentation. The animals were fed on a standard pellet diet and water.

Results:exposure of rats to γ-radiation caused a significant increase in kidney function tests, decreased significantly the antioxidants with numerous histopathological changes in the rat kidney tissue. These changes were ameliorated by bone marrow transplantation either after whole body gamma-irradiation and/or Fe-NTA treatment.

Conclusion: bone marrow transplantation either after whole body gamma-irradiation and/or Fe-NTA treatment restored the kidney functions and ameliorated the oxidative stress and antioxidants markers. The histopathological observations showed amelioration in the structure of the kidney cortex. So, BM transplantation exerts some curative effects on the function and histological structure of kidney cortex of rats exposed to gamma-irradiation and/or Fe-NTA treatment.

Keywords: Gamma radiation, Ferric-nitrilotriacetic acid (Fe-NTA), Bone marrow transplantation, Rats.

INTRODUCTION

Due to the progressive development in all areas of science and technology in the world there are a growing number of various sources of radiations. Those include: mobile communications, development of new methods of medical diagnostics, space exploration, creation of nuclear weapons and the development of the nuclear industry and power that led to a serious threat to the environment and human health [1]. 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 MeV [2]. Whole body gamma-irradiation of animals at the sub lethal and lethal dose levels alters the metabolism of various organs and causes a series of biochemical and physiological disturbances in the different biological tissues. Ionizing radiation produces harmful effects on the organisms and due to the wide spread use of radiation in diagnosis therapy, industry,therefore, pharmacological intervention could be most potent strategy to protect human or ameliorates the deleterious effect of ionizing radiation [3].

Eid *et al.*[4] reported that radiation induced reactive oxygen species(ROS) and free radicals which react with the molecules of cell membranes and induce lipid peroxidation products which play an important role in the biological damage such as mutagenic and carcinogenic damage. They also demonstrated many histopathological and biochemical changes in the kidney tissue post exposure of rats to gamma rays. Ferric-nitrilotriacetic acid **(Fe-NTA)** is a potent nephrotoxic agent and induced acute and sub acute renal proximal tubular necrosis by catalyzing the decomposion of H_2O_2-derived production of hydroxyl radicals, which are known to cause lipid peroxidation and DNA damage [5].

MATERIALS AND METHODS

Experimental animals

In this study, 48 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 condition during the whole

Received: 20/08/2016
Accepted: 28/08/2016
DOI : 10.12816/0029033

period of experimentation. The animals were fed on a standard pellet diet and water.

Animals were categorized into eight main groups; each group was consisted of 6 rats as follows:

G 1: Control group, animals were left without any treatment (four months)

G 2: BM transplanted group, animals control recipient of bone marrow cells group**.**

G 3: R group, animals exposed to 6Gy of γ- rays one month before killing the animals.

G 4: R+ BM group, animals IV injected with bone marrow cells one hour before exposure to 6Gy of γ- rays.

G5: Fe-NTA treated group.6 animals were intraperitoneally injected with a carcinogen compound **(**Fe-NTA) twice a week at a dose level 9 mg/kg body weight **[6]** for four months.

G 6: R+ Fe-NTA group, animals were interprotoneally injected with Fe-NTA twice a week at a dose level 9 mg/kg body weight and exposed to 6Gy of γ- rays one month before killing the animals.

G 7: Fe-NTA+BM group, animals were intraperitoneally injected with Fe-NTA twice a week at a dose level 9 mg/kg body weight and injected with bone marrow cells one month before killing the animals.

G8: Fe-NTA+R+BM group, animals were intraperitoneally injected with Fe-NTA twice a week at a dose level 9 mg/kg body weight, exposed to 6Gy of γ- rays and injected with bone marrow cells one month before killing the animals.

Sample of nearly $1cm^3$ of kidney tissue after the end of the experiment were removed from the dissected animals. Part of tissue was stored in the refrigerator for the biochemical analysis and the other part was fixed in 10% neutral formalin for the histopathological studies. For the histopathological studies, tissue sections were stained by hematoxylin and eosin. stain. For the biochemical estimations, thiobarbituric acid reactive substances (TBARS),reduced glutathione (GSH),glutathione peroxidase (GP), were estimated. Kidney function test urea was detected according to the method of **Tabacco *et al.*** **[7]** and creatinine according to the method of **Young and Friedman** **[8]** .

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.

The biochemical study:

In the biochemical study, thiobarbituric acid reactive substances (TBARS)were estimated as the method of **Yoshioka *et al.*** **[9]** and reduced glutathione (GSH) according to the colorimetric method of **Butler**[10] ***et al.*** **[10].** Glutathione peroxidase activity was measured calorimetrically in plasma and in 10% kidney tissue homogenate as described by the method of **Gross *et al*.**[11]**.**

Kidney function tests:

Enzymatic colorimetric-kinetic method was used for determination of creatinine in plasma was carried out according to the method of **Young and Fridman** **[8]** and urea according to **Tabacco *et al.*** **[7].**

Irradiation technique:

Irradiation was performed by gamma cell 40 (cesium 137) at National Center for Radiation Research and Technology, Exposures to gamma radiation were performed at dose level 6Gy.

Preparation of Fe-NTA solution:

The Fe- NTA solution was prepared by the method of **Gross *et al.***[6]. Briefly, ferric nitrate (0. 16 m moles/kg body weight) solution was mixed with a 4-fold molar excess of disodium salt of nitrilotriacetate (0. 64 m moles/ kg body weight) and the pH was adjusted to 7. 4 with a sodium bicarbonate solution. The solution was prepared immediately before its use.

RESULTS

Biochemical Observation

Kidney function tests:

Creatinine and urea:

Table 1 and Figure 1 represented the levels of kidney function tests in the different experimental animals groups.

Bone marrow transplantation recorded a non significant change either for creatinine or urea levels compared to the control level in rats (Table 3). Exposure of rats to γ-radiation recorded a significant increase in the kidney function tests. The increase was 87.5% for creatinine and 45.8% for urea compared to the control level. On the other hand when the

experimental animals were treated with Fe-NTA the same observation was recorded and it was increased by 112.5% for creatinine and 63.2% for urea.

Combined treatments of rats with Fe-NTA and γ-radiation represented great alterations in the kidney function tests and showed a significant increase in creatinine and urea.

Meanwhile, the treatment of Fe- NTA and /or γ-radiation groups with bone marrow transplantation represented amelioration in kidney functions tests.

Changes in Oxidative stress markers

Lipid peroxidation levels:

For kidney tissue, levels of LPX, GSH and GPX were shown in Table 2.Treatment of rats with bone marrow transplantation represented a non significant change compared to the control group. On the other hand, exposure of rats with γ-radiation or Fe-NTA showed a significant increase in kidney LPX with percent of change 132.9 and 139 respectively ($P \leq 0.05$) and a significant decrease in kidney GSH and GPX levels with percent of change -27.4,-44.8,-60.4, 25.6,-44.1and -52.1 respectively ($P<0.05$) compared to the control level.

However, treatment of the experimental animals with Fe-NTA and γ-radiation (Fe-NTA + R) resulted in a significant increase ($P<0.05$) in LPX level (152.1%) .

Meanwhile, treatment of the experimental animals with Fe-NTA and /or γ-radiation groups with bone marrow transplantation showed a significant decrease in LPX level and showed a significant increase in Kidney GSH and GPX levels compared to the control group level **(Table 2).**

Histopathological Observations.

Normal structure of kidney cortex is shown in Figure 2. The circular areas observed in this photograph are the renal Malpighian corpuscles. Each one is composed of glomerulus surrounded by Bowman's capsule with thin glomerular basement membranes and patent capsular space. Numerous tubules (proximal and distal) lie in the area adjacent to the glomeruli. A section in rat kidney treated with BMc transplantation showed no detectable changes either in Bowman's capsules, Malpighian corpuscles and distal or proximal convoluted tubules (Figure 3).

Rats exposed to γ- radiation showed areas of bleeding, lesions and lobulated glomeruli. On the other hand, many of the convoluted tubules have a pyknotic nuclei (Figure 4). However, treatment of irradiated animals with BMc transplantation reduced bleeding areas, lesions with normalization of the convoluted tubules (Figure 5).

Treatment of rats with Fe-NTA showed highly atrophied, congested and lobulated glomeruli, accumulation of inflammatory and bleeding lesions in the kidney cortex (Figures 6,7). Reduced damage of Fe-NTA was realized in rat's kidney cortex when experimental animals were treated with BMc transplantation, but lobulated glomeruli were still detected (Figure 8). Appearance of congesteed glomeruli and bleeding lesions in addition to the presence of marginal chromatin in some cells of distal convoluted tubules with debris of ruptured cells (Figure 9 A&B) were observed when rats were treated with Fe-NTA and exposed to γ-rays. However, treatment of the previous group (Fe-NTA+γ-rays) with BMc transplantation showed atrophoid glomeruli and accumulation of inflammatory cells surrounding Bowman's capsules (Figure 10).

DISSCUSION

Humans on earth are exposed to many sources of ionizing radiations. The largest component of man-made background radiation relates to exposures associated with medical diagnosis and treatments. Clinical and pathological studies revealed that radiation therapy can produce significant tissue injury [12]. On the other hand, bone marrow cells suppress immune cell responses and have beneficial effects in various inflammatory-related immune disorders. [13] .

Ferric nitrilotriacetate (Fe-NTA) is a known potent nephrotoxic agent.

In the present study, treatment of Fe- NTA and /or γ-irradiation groups with bone marrow transplantation showed amelioration in kidney, Fe-NTA injected rats indicated general toxicity that occurred due to renal injury. Kidney plays important roles in blood filtration and excretion of waste products and reabsorption of nutrients, thus serving as the main organ for maintaining homeostatic conditions [14].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 lipid peroxidatin and kidney tissue compared to the untreated groups. **Maha *et al*.** [15] found that in the irradiated animals received BMT significantly depressed lipid peroxidation was realized in the serum and tissues 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 glutathione , glutathione peroxidase levels in the blood, kidney tissues as compared to the Fe-NTA and /or γ-irradiation untreated group.

In the present study, whole body exposure of rats to 6 Gy induced alteration in creatinine and urea levels markers of kidney function compared to the control values. Elevation in serum urea and creatinine levels may result from reduced activities of several enzymes that play a role in renal function as well as reduced adenosine triphosphate production due to uncoupling of the mitochondrial oxidative phosphorylation [16].

The recorded increase in MDA could be explained on the basis that ionizing radiation induces lipid peroxidation through the production of reactive oxygen species, which attack the polyunsaturated fatty acids of the phospholipids of the cell membrane [17].

The decreased antioxidant levels in γ- irradiation group may be due to their utilization by the enhanced production of ROS [18 &19] .

Radiation exposure induces radiolysis of water in the aqueous media of the cells which leads to production of hydroxyl radicals (•OH). Hydroxyl radicals interact with the polyunsaturated fatty acids in the lipid portion of biological membranes initiating the lipid peroxidation and finally damage the cell membranes [20]

Liu *et al.* [21] observed a significant decrease in levels of kidney Caspase 3 in group subjected to Fe-NTA. Caspase 3 seems to play an important role in Fe-NTA-dependent loss of cell viability because a relatively specific peptide inhibitor of caspase 3. Fe-NTA induced ROS, which play a protective role in apoptosis by inhibiting Caspase-3 activation. Fe-NTA-induced ROS increased mRNA and protein level of anti-apoptosis Bcl-2 and decreased mRNA protein level of pro-apoptosis gene Bax, As a result, maintaining mitochondrial membrane potential [21].

Kidney cortex of rats exposed to 6 Gy gamma radiation showed bleeding lesions, lobulated glomeruli and many of the convoluted tubules have pyknotic nuclei. PI/AO stain showed many necrotic areas and some underwent apoptosis in the convoluted tubules .Many of the convoluted tubules showed protrusion in its lining cells, obstructed or completely degenerated. Microscopic studies in rats exposed to 6 Gy of gamma radiation showed a similar sequence of glomerular injury with tubular alterations [22].

According to **Iqba *et al.*** [23]. Treatment of rats with Fe- NTA showed highly atrophied, congested or lobulated glomeruli were detected with accumulation of inflammatory and bleeding lesions in kidney cortex these findings are supported by several studiesFe-NTA is known as a complete renal carcinogen as well as renal and hepatic tumour promoter, acting by inducing reactive oxygen species generation in the tissues**. Toyokuni *et al.*** [24] reported that all four DNA bases are modified in the renal chromatin of rats within 24 h of Fe-NTA treatment. These lesions are typical products of hydroxyl radical reactions. The ability of Fe-NTA to promote DNA and membrane damage has been postulated as a critical mechanism in renal carcinogenesis associated with the intraperitoneal injection of this complex.

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Mona and Mervat [25] studied 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, In kidney tissue one of the most significant reports of bone marrow derived cells contributing to renal repair is that of **Cornacchia *et al.*** [26] who demonstrated that bone marrow from mice with an inherited glomerular mesangial sclerosing defect transferred the disease phenotype, the morphology and matrix metalloproteinase expression levels were due to generation of endothelial and mesangial cells from the donor bone marrow. 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 **(Hall *et al.*, 2001)**[27].

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Table 1 - Effect of bone marrow transplantation on kidney functions tests of Fe-NTA and /or γ-radiation treated rats

Parametres Groups	Creatinine (mg/dl)	Urea (mg/dl)
G1: Mean±SE	0.8±0.05	32.1±0.7
G2: Mean±SE % of change from G1	0.6±0.04bc -25%	30.9±0.8bc -3.7%
G3: Mean±SE % of change from G1	1.5±0.06ac 87.5%	46.8±0.6ac 45.8%
G4: Mean±SE % of change from G1	1.7±0.07ab 112.5%	52.5±0.8ab 63.2%
G5: Mean±SE % of change from G1	2.1±0.09abc 162.5%	58.1±0.9abc 80.9%
G6: Mean±SE % of change from G1	1.3±0.06abc 62.5%	40.8±0.8abc 27.1%
G7: Mean±SE % of change from G1	1.1±0.08abc 37.5%	36.9±1.2abc 12.2%
G8: Mean±SE % of change from G1	1.0±0.07bc 12.5%	35.7±1.3bc 8.5%

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.

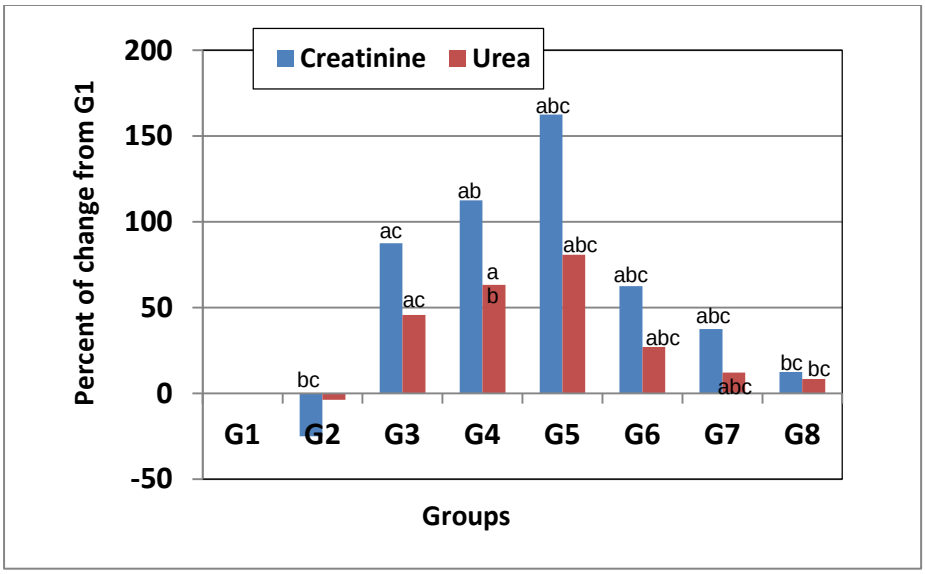

Figure 1- Percent of change in kidney function tests of Fe-NTA and /or γ- irradiated rats treated with bone marrow transplantation.

Table 2- Effect of bone marrow transplantation on LPX , GSH and GPX levels of blood and tissue organs of Fe-NTA and /or radiation treated rats

Kidney Tissues **Groups**	**LPX** (µM/g wet tissue)	**GSH** (µM/g wet tissue)	**GPX** (µmol/min/ g wet tissue)
G1: Mean±SE	88.9±2.2	90.1±2.6	156.5±1.9
G2: Mean±SE % of change from G1	85±1.4^{bc} -4.4%	88.8±2.2^{bc} -1.5%	154.5±2.6^{bc} -1.3%
G3: Mean±SE % of change from G1	132.9±1.3^{a} 49.5%	65.4±2.4^{ac} -27.4%	116.5±2.1^{ac} -25.6%
G4: Mean±SE % of change from G1	139±2.1^{a} 56.4%	49.7±3.2^{ab} -44.8%	87.5±1.9^{ab} -44.1%
G5: Mean±SE % of change from G1	152.1±1.9^{abc} 71.1%	35.7±2.2^{abc} -60.4%	74.9±1.9^{abc} -52.1%
G6: Mean±SE % of change from G1	109±2.8^{abc} 22.6%	74.5±1.9^{abc} -17.3%	120.4±2.7^{abc} -23.1%
G7: Mean±SE % of change from G1	112.3±3.1^{abc} 26.3%	78.6±1.6^{abc} -12.8%	118.3±2.2^{abc} -24.4%
G8: Mean±SE % of change from G1	91.3±1.9^{bc} 2.7%	88.9±3.8^{bc} -1.3%	139.2±3.7^{abc} -11.1%

Legends as in Table (1)

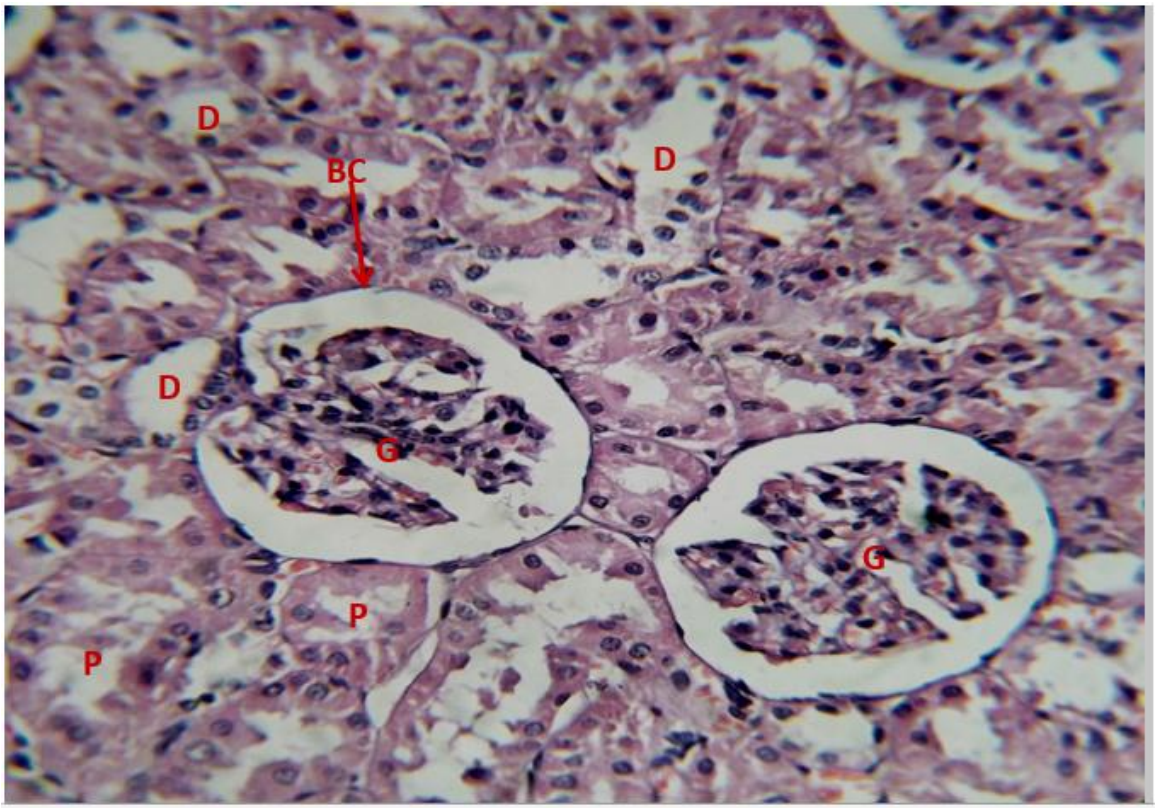

Figure 2- Photomicrograph of a section of kidney cortex of a control rat shows Bowman's capsules (BC), Malpighian corpuscles, distal (D) and proximal (P) convoluted tubules. (H&E stain X400)

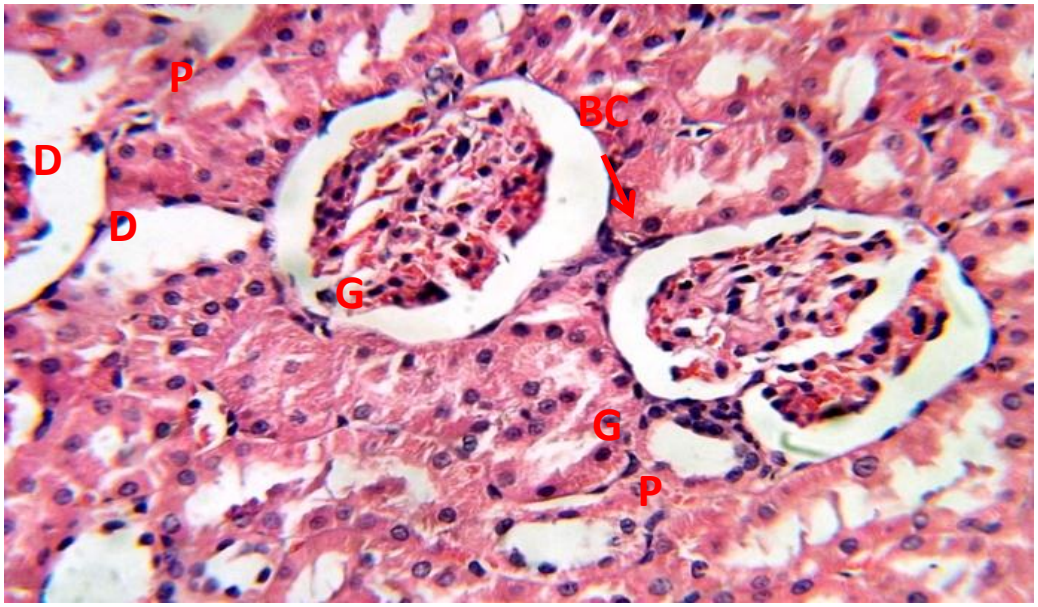

Figure 3- Photomicrograph of a section of kidney cortex of rat treated with BMc transplantation shows no detectable changes in Bowman's capsules (BC), Malpighian corpuscles, distal (D) or proximal (P) convoluted tubule.. (H&E stain X 400)

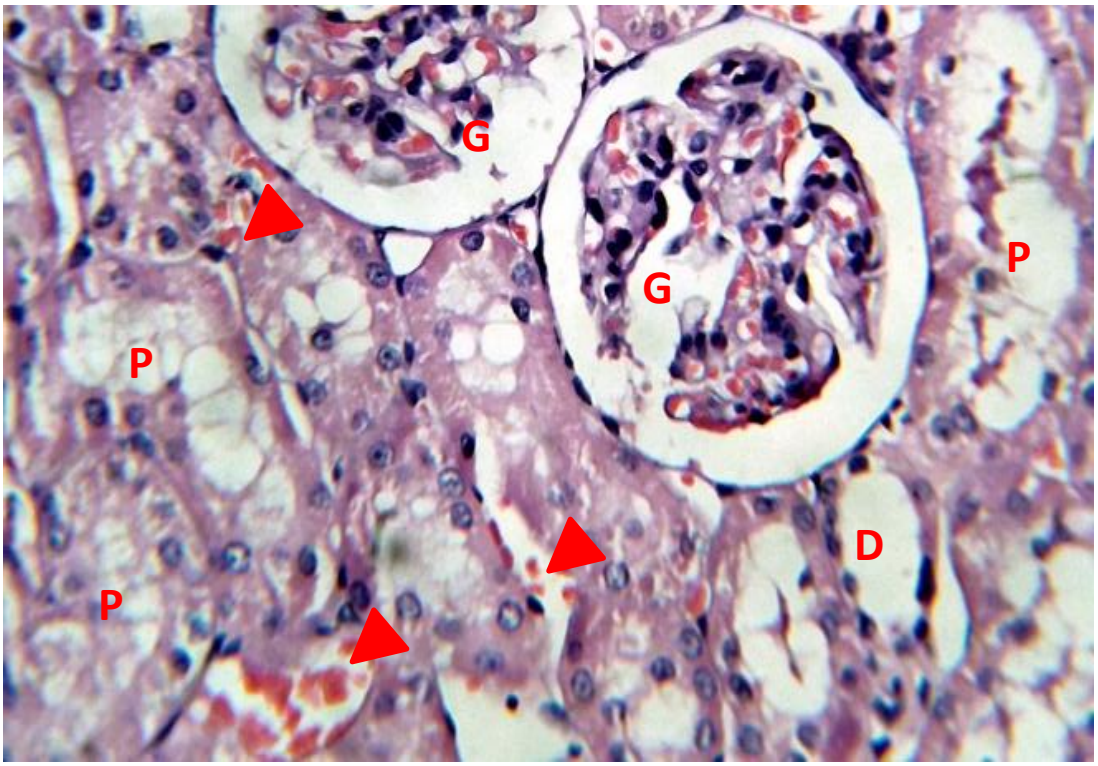

Figure 4 - Photomicrograph of a section in rat kidney cortex of a rat exposed to γ- radiation shows the appearance of bleeding (▲), lesions and lobulated glomeruli. Notice many of the convoluted tubules (P&D) which have pyknotic nuclei. (H&E stain X400)

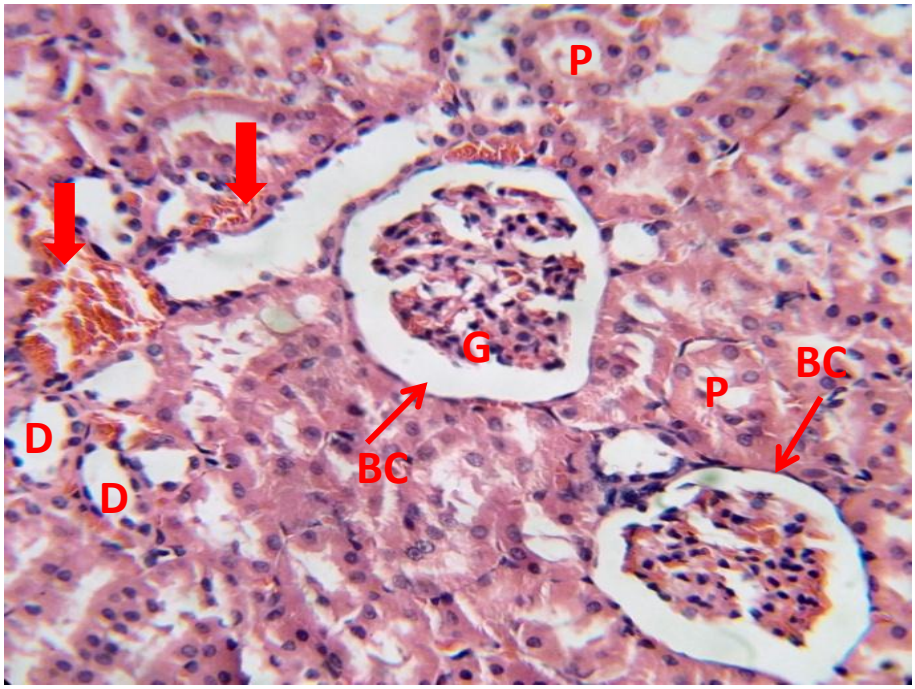

Figure 5- Photomicrograph of a section of kidney cortex of a rat exposed to γ- radiation and treated with BMc transplantation shows few areas of bleeding (red blocked arrows) with normalization of convoluted tubules. (H&E stain X400)

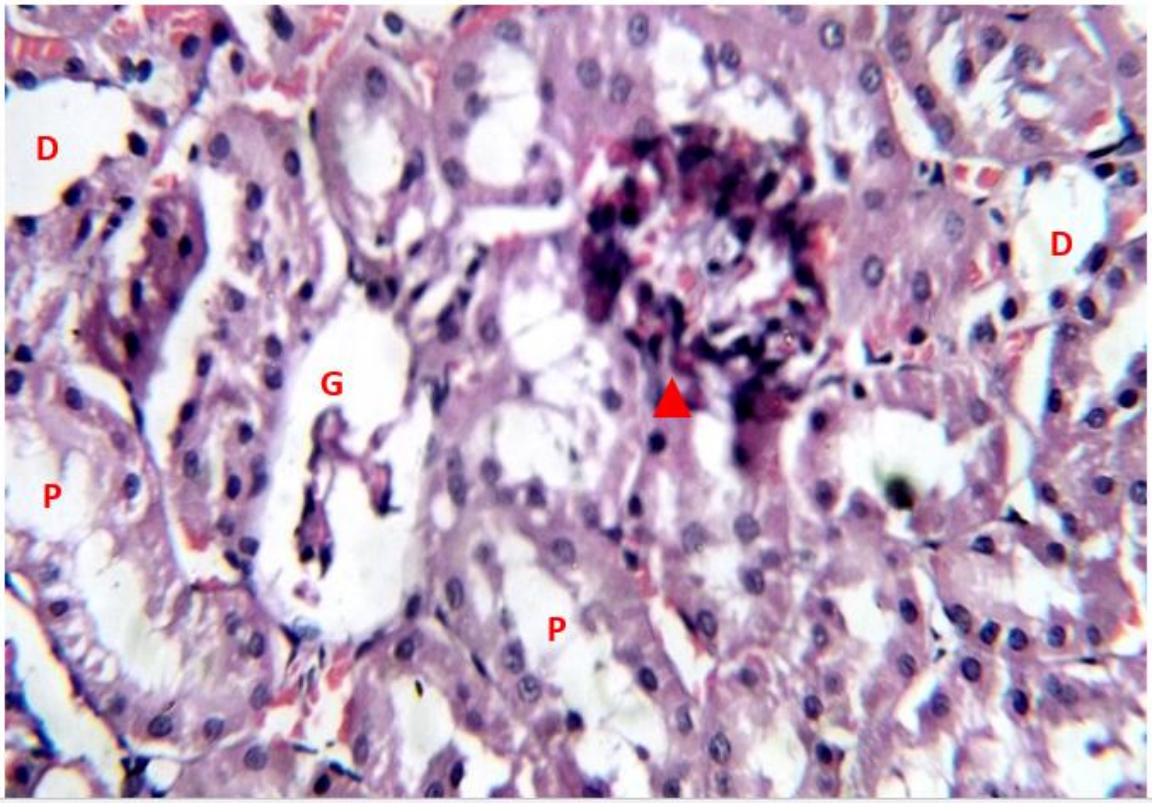

Figure 6- Photomicrograph of a section in rat kidney treated with Fe-NTA recording highly atrophied glomeruli (**G**) and accumulation of inflammatory cells (▲).

(H&E stain X400)

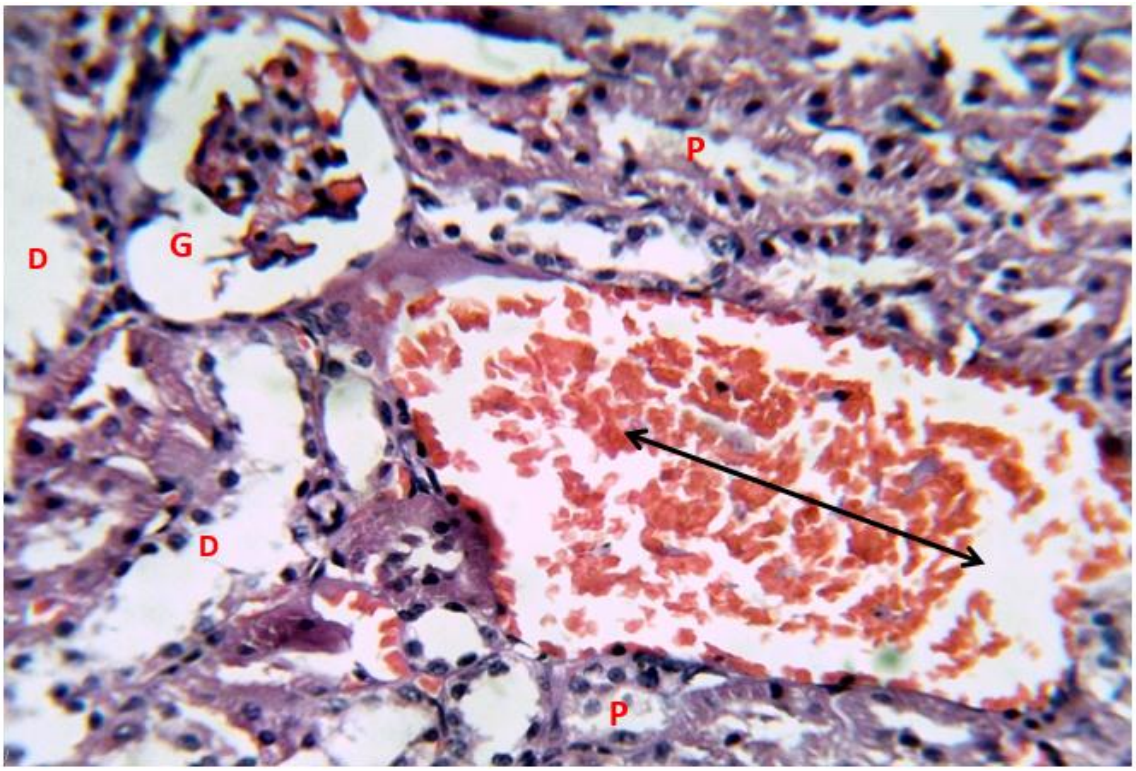

Figure 7- Photomicrograph of a section in rat kidney treated with Fe-NTA shows large and small areas of bleeding (↕), congested, lobulated and atrophied glomeruli (G). (H&E stain X400)

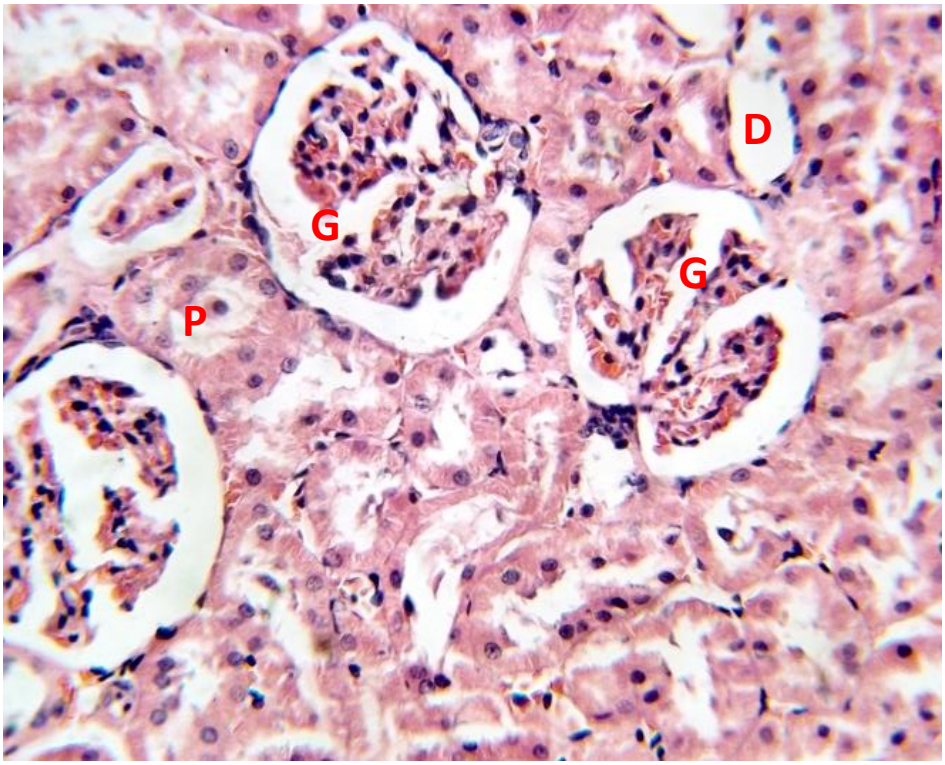

Figure 8- Photomicrograph of a section in rat kidney treated with Fe-NTA and BMc transplantation shows lobulated glomeruli (G). (H&E stain X400)

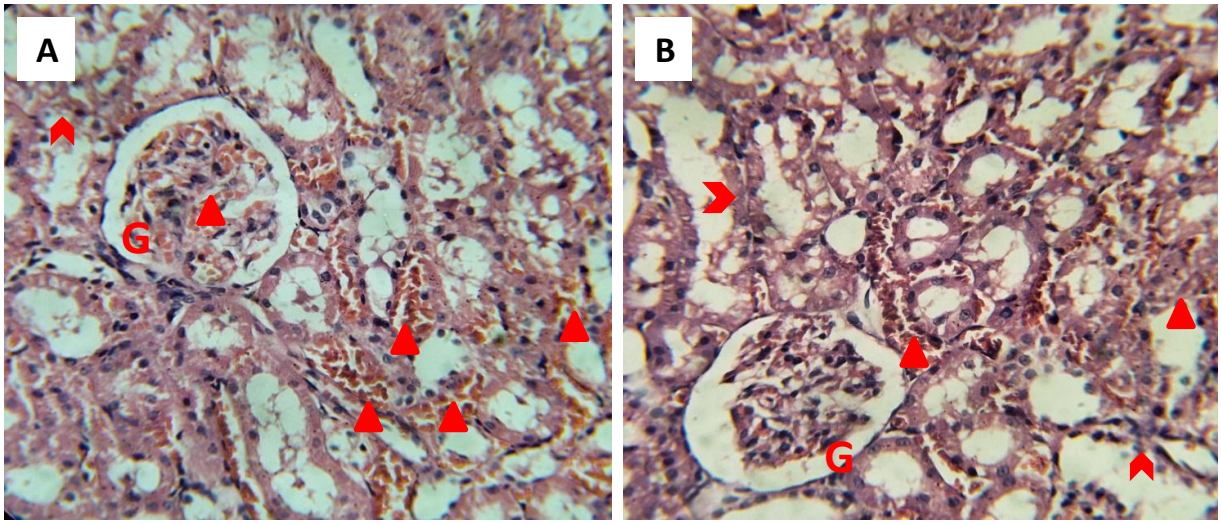

Figure 9.A&B- Photomicrograph of a sections in rat kidney treated with Fe-NTA and exposed to γ-rays bleeding areas (▲) in addition some of the distal convoluted tubules cells contain pyknotic or karyolytic nuclei with debris of ruptured cells (^). (H&E stain X 400)

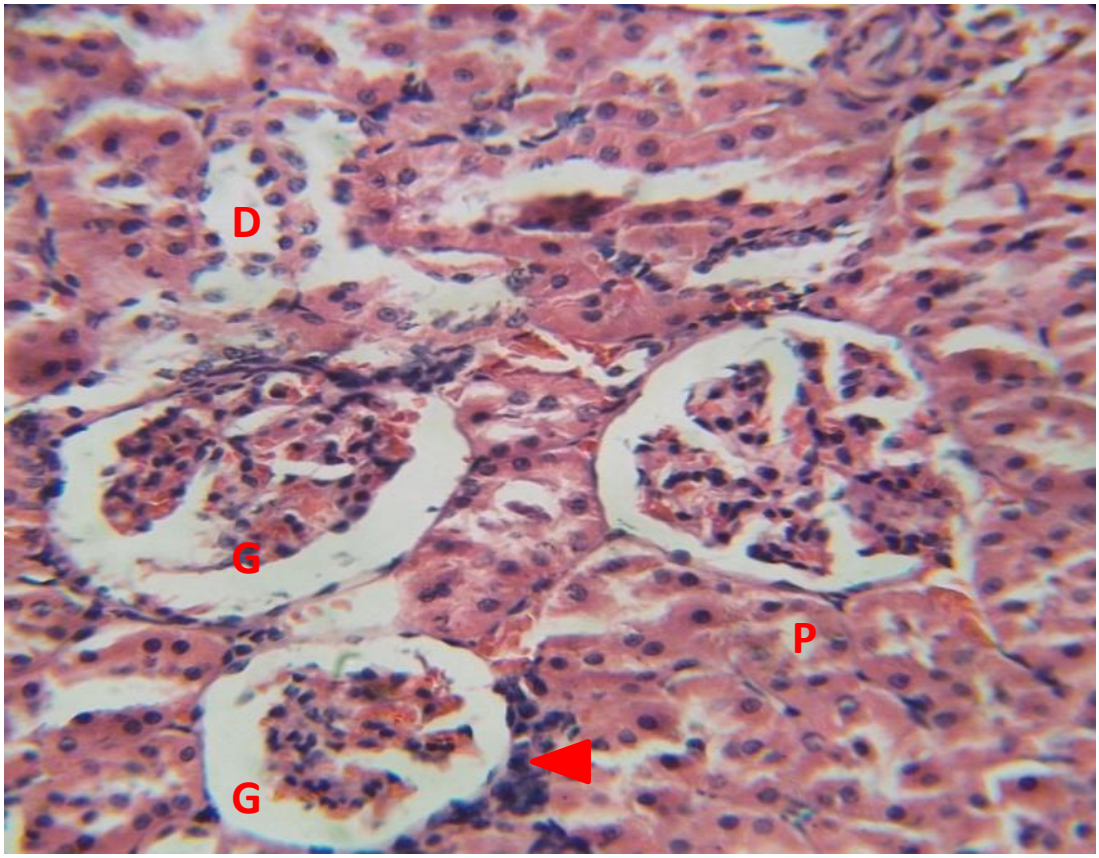

Figure 10- Photomicrograph of a sections in rat kidney treated with Fe-NTA, exposed to γ-rays and treated with BMc transplantation shows appearance of atrophoid glomeruli (G) and aggregated inflammatory cells surrounding Bowman's capsules ( ). (H&E stain X 400)