PASSIVE EFFECT OF X-RAY IRRADIATION ONTESTICULAR FUNCTION, SPERMATOGENESIS, SOME BLOOD PARAMETERS AND TESTOSTERONE IN MALE RABBITS

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ABSTRACT

The present study was done to determine and evaluate the effect of X-ray irradiation on the testicular tissue of rabbits those were exposed for a long time. Ten male rabbits, 8-9 months old and their weight approximately two kg. Rabbits were exposed to X-ray irradiation for two months/ twice daily. Blood parameters and testosterone hormone were measured within 20th, 40th, and 60th days after exposure. Orchictomy were done by surgical methods after 60th days for histopathological examination. The results revealed highly changes in testis such as atrophy, hyper atrophy, blood vessel congestion and suppression of spermatogenesis, blood parameter also changed and testosterone levels reach to zero at 60th days after exposure. In concluding that the persistence of X-ray exposure caused deterioration and passive effects on testicular tissue and other organs of rabbits.

INTRODUCTION

Hazardous irradiation of x-ray is important to the patient and technician safety (1). Radiation effects on the brain tissue are cause typical necrosis and congestion in (2), physiological changes also show in serum glutathione (GSH), sulphydry [5,5dihiobis-2-nitrobenzoic (DTNB)] and serum protein concentration as well as lipid profile, these changes tend to increase (3).X-ray irradiation effects on the stomach cause gastric disorders which observed gastric erosion, ulcerative gastritis, stomach dilatation, and gastroparesis (4). The liver is sensitive to X-ray irradiation, the researchers showed primary liver cancer, large number of blood vessels as well as elevated liver enzyme parameters (5, 6). The risk of x-ray to the pancreas, heart, eye,

and other body organs are very dangerous which cause pancreatic tumor, pancreatic damage, eye lens cataract, and tachycardia (7,8). Male reproductive system in rabbits and other animals are sensitive to x-ray irradiation (9). The male reproductive system in rabbit consists of pair testis, epididymis, ampoules, vas deferens, urethra, penis, glands and accessory glans (10), but the sensitive organ in the male reproductive system is the testis and neighbor organs (11). X-ray irradiation induces rapid germ cell division and cause DNA damage due to long prophesied and meiotic division to rise to haploid spermatids during this phase, the chromatin of DNA is extensive and are replaced by basic transition proteins followed by proteins (12). Histochemistry of testicular tissues has also changed with x-ray irradiation, when tests under 300 race, ascorbic acid concentration and cholesterol of testes are decreasing sharply, alkaline phosphatase is elevated (13). Histopathological distinct change, spermatozoa are completely depleted, dense irregular nuclei, and acidophilus vaculated cytoplasm, number of Sertoli and Leyding are reduced by 70 % of the total number (14). Somniferous tubules are completely damaged and spotted with the connective tissue of Levding cells (15). Due to destruction of testicular tissue the metabolism will be decreased, therefore the scavenger cell of free radical reduced, mutagencity and develop the tumorous cells advertisement increase (16).

MATERIALS AND METHODS

Ten rabbits were used in the present study, their were weight 2 ± 0.05 kg, their ranged between age 8-9 months. Clinically, the rabbits were examined to ensure the health of the testicles and sexual behavior. The testicles were measured by Vernia before irradiation exposure. Irradiation exposure by using X-ray machine[Ecoray-USA, k. v=70, current=200mAmper, time=70 second] figure [1, 2] (17). The rabbit testicles were irradiated by X-ray under general anesthesia by ketamine 13mg/kg.bw+ xylazine 5mg/kg.bw combination (18), the exposure for two months, twice per day directly. After two months the rabbits were castrated by open surgical method castration figure [3,4] (19). Blood collection were done by heart direction puncture with sterile syringe. Macroscopic images were taken over the operation and histopathological were made slides and stained by E & H stain technique (20).

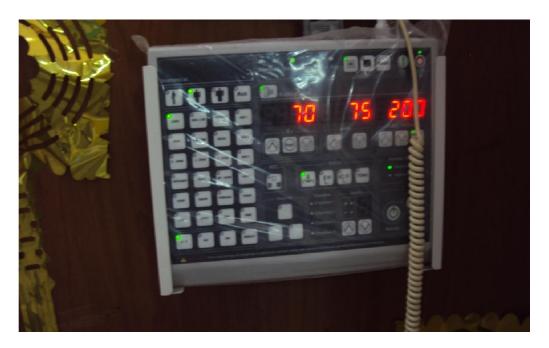


Fig (1) ;X-ray board control. k.v= 70, time=75, current=200 mAmper



Figure (2); Testicle preparation for Castration

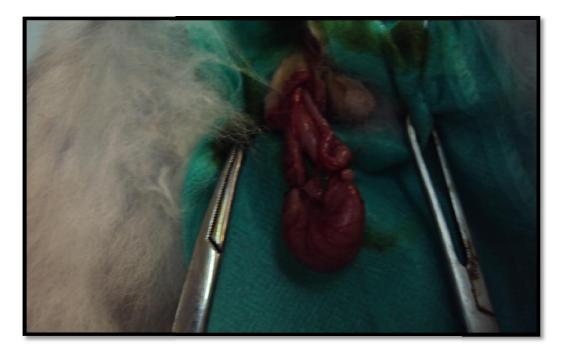


Fig. (3); Testicle Castration by open surgery

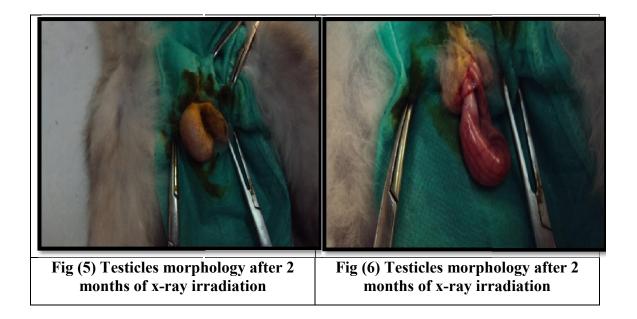
RESULTS

1-Clinical and behavioral symptoms

Healthy status of animals is good and depression, sexual behavior is non-existent, but they healthy and normal eating and drinking as well as walking.

2-Gross finding

Gross pictures of rabbit testis were enlarged and edematous and smootherappearance, but after sectioning, the congestion and enlargement of superficial veins. Show picture (1 and 2)



3-Microscopic finding

Histopathological picture of testis after two months of exporter to x-ray were shown a different lesion due to X-ray irradiation. Edema is prominent in most slides. Somniferous tubules in all animals testis were destroyed and separation, blood vessels was congested and another hemorrhage while other vessel wall is thickened, fibrosis were showing in tubules and interstitial tissues. Necrosis, inflammatory cells and fibrocytes were invasion whole testis of rabbits, show picture (3,4.5,6,7,8,9 and 10).

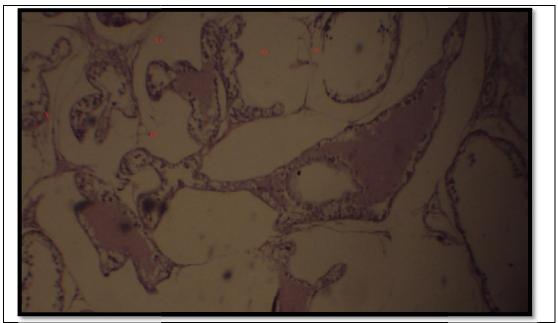


Fig. (7); Rabbit testis histopathology after two months of x-ray irradiation edema into interstitial cells (O), Atrophy of somniferous tubules and suppression spermatogenesis(A). E&H staining 100X

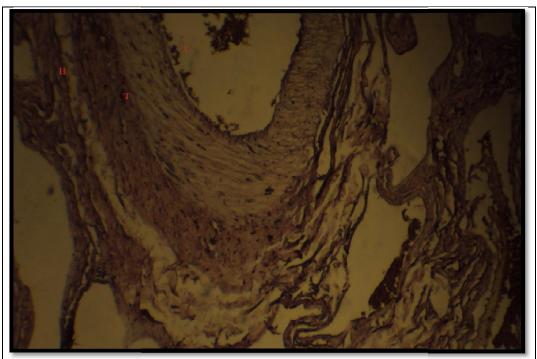


Fig. (8) Rabbit testis histopathology after two months of x-ray irradiation congestion of blood of vessels(C), hemorrhage (H), thickening of blood vessel wall (T). E&H staining 100X

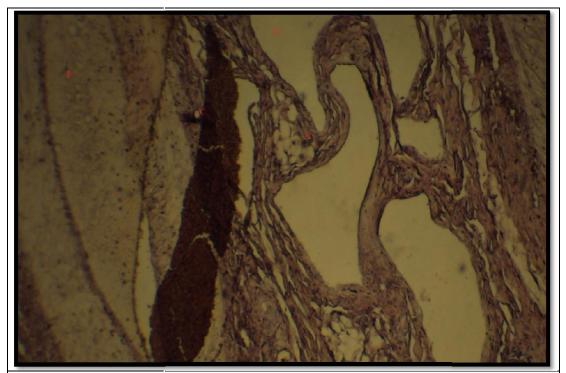


Fig. (9) ;Rabbit testis histopathology after two months of x-ray irradiation congestion C, edema A and fibrosis F. E&H staining 100X

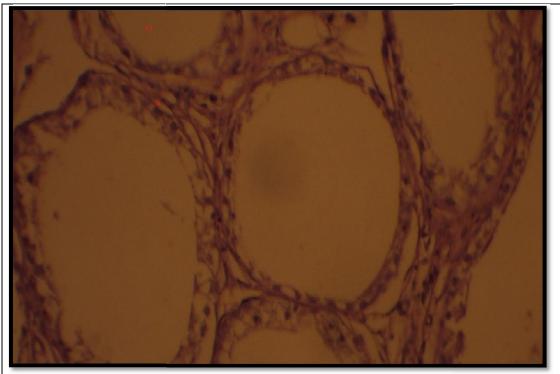


Fig. (10); Rabbit testis histopathology after two months of x-ray irradiation vacuolated of the somniferous tubules lining and fibrosis as well as fibrocytes. E&H staining 100X

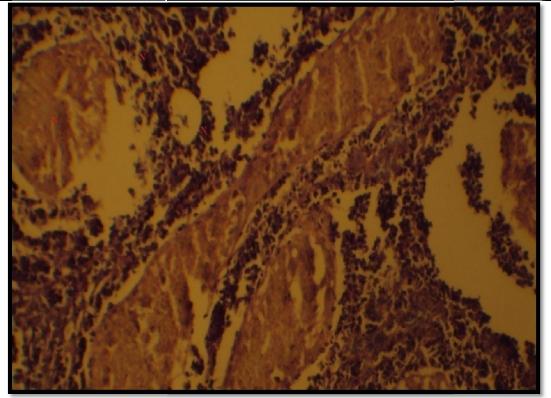


Fig. (11); rabbit testis histopathology after two months of x-ray irradiation there are hemorrhage, necrosis N and excessive amount of inflammatory cells. E&H staining 100X

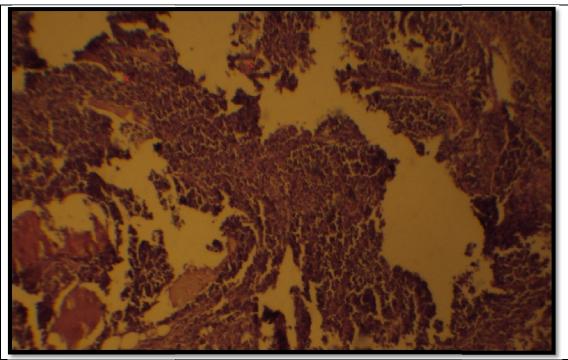


Fig. (12); Rabbit testis histopathology after two months of x-ray irradiation excessive amount of inflammatory cells and necrosis N. E&H staining 100X

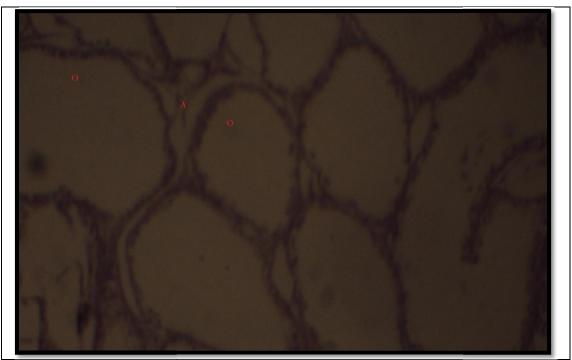


Fig. (13); Rabbit testis histopathology after two months of x-ray irradiation edema into interstitial cells atrophy of somniferous tubules, suppression of seminefrous tubules . E&H staining 100X



Fig. (14); rabbit testis histopathology after two months of x-ray irradiation fibrosis Se and thickening of semineferous wall and supervision of spermatogensis. E&H staining 100X

4-Blood profile and hormone estimation

Blood parameters and testosterone /period	1 st day before x- ray exposure	After 20 days of x-ray exposure	After 40 days of x-ray exposure	After 60 days of x-ray exposure
WhiteB C*10 ⁹	7.0 ±2.1*	7.2±1.1	9.2±1.6	$11.2 \pm 1.6^{**}$
Neutrophils *10 ⁹	3.6 ±1.5	3.8±5.5	4.8 ±0.5	7.8 ±0.5
Lymphocytes *10 ⁹	2.5 ±1.1	3.5 ±1.1	3.5 ±0.1	4.5 ±0.1
Monocytes*10 ⁹	2.40 ± 0.1	2.90 ± 0.8	4.90 ± 0.8	4.90 ± 0.8
Eosinophils*10 ⁹	0.03 ± 2	1.03 ± 2.7	1.63 ± 2.7	$2.23 \pm 2.7^{**}$
Basophils*10 ⁹	0.001 ± 2	0	0	0
BRC*10 ¹² /L	5.5 ±0.3	5.1 ±0.2	4.1 ±0.7	3.1 ±0.7
Hemoglobin(g/dl)	11.5±0.8	10.2±0.1	8.2±0.1	6.1±0.1**
Platelets*10 ⁹	201-716	101-615	101-415	78–215**
Testosterone (n/dl)	3.25±1.52	1.29±1.62	0.29±1.62	$0.01 \pm 1.02^{**}$

Table (1) blood profiles and testosterone in different times

*Mean and standard deviation, P value ≤ 0.05

Statically Analysis

The data were analyzed statically by used SPSS 18.0 program. Normal values P value ≤ 0.05 , the significant values ≤ 0.005 .

DISCUSSION

Clinical symptoms of healthy rabbits excepts abnormal size of testis is not necessarily the rabbits don't suffer from disorders, but our study didn't include biochemical or medicinal abnormalities. Gross morphology of testis in all experimental animals revealed testicular atrophy and other hypertrophy, this phenomenon was contradictory, and was explained several possibilities, the X-ray irradiation was affected the renal system which cause associates and testicular edema in single testicular rand don't effect to other testicular (21), other probability because of liver disorders due to X-ray irradiation, most researchers noticed effect in the effect testis (22), other probability may be cardiac disorder and cardiovascular failure also cause interstitial edema and testicular hypertrophy (23). Another aspect of the testis was atrophy due to direct exposure of X-ray because the test is somniferous tubules cause testicular dysfunction and effects in the testicular atrophy (24). Microscopic images had different patterns of histopathological changes such as somniferous tubules, hemorrhage, thinning of blood vessels and congestion reveal to the highly negative effect of X-ray irradiation, and the mechanism of effect of cellular ionization by X-ray, this ionization interfere with cells bioactivity and cause different histopathological changes in testis. Blood profile ordinary also changed due to the nature of x-ray irradiation physics, physics of x-ray is electromagnetic and cause blood magnetism lead to iron liberation from red blood corpuscles, therefore the present study shown declined showed RBCs count after 60 days of x-ray irradiation as well as the hemoglobin also decrease for the same reason (4). Complete WBCs and differential WBCs showed graduate increase due to tissue destruction specially testis, testosterone hormone sharply decreased after 60 days of x-ray exposures due to complete layding cells destruction which responsible testosterone secretion (25).

التأثير السلبي لإشعاع الأشعة السينية على وظائف الخصية وتكوين النطف، وبعض مكونات الدم والهرمون الذكري في ذكور الأرانب

أحسان علي حبيب ، إبر اهيم محمد حسن الراشد، لؤي احمد نعيم ، زينب بكر عبد الكريم فرع الجراحة والتوليد، كلية الطب البيطري ، جامعة البصرة [،] البصره ،العراق

الخلاصة

هدفت وقيمت الدراسة الحالية تأثير إشعاع الأشعة السينية على أنسجة الخصية وأعضاء أخرى في الأرانب التي عرضت لفترة طويلة للأشعة السينية، استخدمت 10 أرانب ذكور ذات عمر من 8-9 اشهر تزن تقريبا 2 كيلوغرام، عرضت الى الأشعة السينية بجرعة 75 راد/م²، مرتين باليوم لمدة شهرين، فحصت عينات الدم وهرمون الذكري في الفترات 20، 40، و60 يوم من بدء الإشعاع، تم خصي الارانب بعد 60 يوم جراحيا، لوحظت التغيرات العيانية على حجم الخصية كالضمور والتضخم والاحتقان الدموي كما لوحظ هناك تغيرات نسجية مرضية لهذه الخصيات واهمها تحطم القنوات المنوية وانسجة اخرى، في الاستنتاج نؤكد ان الاشعاع بالاشعة السينية لفترة طويلة ذات تأثير سلبي على انسجة الخصية والأعضاء الاخرى.

REFERENCES

- 1-Luke S, Kiszel Z, Buschmann J, and Trott, K R (1985). Radiation induce heart disease in rats.Inter.J.Rad. Onco.Bio. Phys., 11(4):891-808.
- 2-Gurney JG, Ness KK, Stoval M, Woden S, Punyko JA, Neglia JP, Mertens AC, Packer RJ, Robison LL, and Sklar CA (2003). Final weight and body mass index among adult survivors of childhood brain cancer: childhood cancer survivor study. J. Clin. Endo. Meta. ,88(10):4731-4739.
- 3-Al-Bazii WJ and Al-Bazii SJ (2014). Histological and physiological study about effect of chronic X-ray exposure on male brain. J. Kerb. Uni., 12(1): 228-234.
- 4-Breiter N, Trott K-R, and Sassy T (1989). Effect of X-ray irradiation on the stomach of the rat. Inter.J.Rad. Onco.Bio. Phys., 17(4):779-784.
- 5-Lee EW, Tafti BA, Prieto V, Totochy M, Hilton J, Dry S, Cho S, Loh CT, and Kee ST (2012). Irreversible electroporation in eradication of rabbits VX2 liner tumor. J VascIntervRadiol., 23(6): 833-838.
- 6-Leible SA, Pajak TF, Massullo V, Order SE, Komak RU, Chang CH, Wasseman TH, Phillips TL, Lipshut ZJ, and Durbin LM (1987). A comparison of misonidazole sensation radiation therapy to radiation therapy alone for the palliation of hepatic metastases: results of a radiation therapy oncology group randomized prospective trial. Int. J. Radia. Onco. Bio. Phys., 13(7): 1057-1066.

- 7-Fujita M, Otsuka Y, Yamada S, Iwakawa M, and Imai T (2011). X-ray irradiation and rho-kinase inhibitor additively induce invasiveness of the cells of the pancreatic cancer line, MIAPaCa-2, which exhibits mesenchymal and ameboid motility. JCA, 102(4): 792-798.
- 8-Pandolfo L, Livrea MA, and Bono A (1986). Effect of bendazacL-lysine salt on Xray induced cataract in the rabbit lens. Exp. Eye Res., 42(2): 167-175.
- 9-Lepekhin NP, and Palyga GF (1994). Consequences for the intrauterine development of the offspring of the irradiation of male germinal cells at different stages of spermatogenesis. Radiat. Biol. Radioecol, 34(4) 645-649.
- 10-Campos AN, GadelhaCR, Guerreiro MR, Pereira ES, Lima ICS, Linard MB, Meneses HM, Castelo KF, and Estevan FN (2014). Male rabbit reproductive physiology. Standard Res. J. Agri. Scie. ,2(8): 120-128.
- 11-Georgieva S, Georgieva P, Tanchev S, and Zhelyazkov E (2005). Effect of external gamma irradiation on rabbit spermatogenesis. T. J. Scie., 4(1): 22-26.
- 12-Cardelli E, Eleuteri P, Grollino MG, Benassi B, Blandino G, Cecilia B, Pardini MC, DiCaprio EV, Spano M, Pacchierotti F, and Villani P (2012). Direct and delayed X-ray induced DNA damage in male mouse germ cells. Envi. Mole. Muta., 53:429-439.
- 13-Santra KB and Manna CK (2009). X-ray induced changes in biochemical and histochemical parameters in the testis of male wild Indian house rat *Rattusrattus*. Cey. J. (bio.Scie), 38(2): 39-49.
- 14-Hussein MR, Dief EE, Ghait AT, Adly MA, and Abdelraheem MH (2006). Morphological evaluation of the radioprotective effect of melatonin against X-ray induced early and acute testis damage in albino rats: an animal model. Int. J. Path., 78:237-250.
- 15-Mornjakovic R, Alicelebic S, Bilalovic N, Susko I (1998). Morphometeric characteristic of the leading cells after total irradiation of rats treated with melatonin. Med. Arb. 52:183-184.
- 16-Take G, Erdogan D, Helvacioglu G, Goktas G, Ozbey G, Uluoglu C, Yucel B, Guney Y, Hicsonmez A, and Ozkan S (2009). Effect of melatonin and time of administration of irradiation induced damage to rat testis. Bar. J. Med. Bio. Res., 42: 621-628.
- 17-Sener G, Jahovic N, Tosun O, Atasoy BM, Yegen BC (2003). Melatonin ameliorates ionizing radiation induced oxidative organ damage in rat. Life Scie., 74: 563-572.

- 18-Fish RE,Biown MJ, Danneman PJ and Karas AZ., . (2008). Anesthesia and analgesia in laboratory animal ^{2nd},American collage of laboratory series, 420-421.
- 19-Fossum T W. Small animal surgery. 4thedu, Elsevier, USA. 2013; 370-375.
- 20-LunaL G (1968). Manual of Histological Staining MethodsBlackstone Division, McGraw-Hill, 45-77.
- 21-Kelemen LE, Cerhan JR, Lim U,(2006). Vegetables, fruit, and antioxidant-related nutrients and risk of non-Hodgkin lymphoma: a National Cancer Institute-Surveillance, Epidemiology, and End Results population-based case-control study. Am J Clin Nutr.;83(6):1401-10.
- 22-Jeggo P. (2010). The role of the DNA damage response mechanisms after low-dose radiation exposure and a consideration of potentially sensitive individuals. Radiat Res.;174(6):825-32.
- 23-Coppes RP, Stokman MA(2011). Stem cells and the repair of radiation-induced salivary gland damage.*Oral Dis.*; 17(2):143-53.
- 24-Bolderston A, Lloyd NS, Wong RD(2006). The prevention and management of acute skin reactions related to radiation therapy: a systematic review and practice guideline. *Support Care Cancer*.;14(8):802-17.
- 25-Wan XS, Ware JH, Zhou Z, Donahe JJ(2006). Protection against radiation-induced oxidative stress in cultured human epithelial cells by treatment with antioxidant agents. Int J Radiat Oncol Biol Phys.;64(5):1475-81