Journal of Kerbala University , Vol. 12 No.1 Scientific . 2014

Histological and physiological study about effect of chronic x-ray exposure on male rabbit brain

دراسة نسجية ووظيفية عن تاثير التعرض المزمن للاشعة السينية في دماغ ذكور الارانب

AL-Bazii, W,J * and AL-Bazii , S.J**
*Kerbala university applied medical science college ,environmental health department **pure education science biology department

Abstract

The aim of this study was to evaluate the effect of x-ray exposure on some physiological parameters and pathological lesions in the brain on male rabbits .

Twenty male rabbits were randomly divided into two groups (10/group) control(C) and group exposed to 100kilorad (Cx) .After three months was showed there was a significant increase($p<0.05$) in the Malondialdehyde (MDA)level and Ach esterase activity , significant decrease ($p<0.05$) in reduced glutathione (GSH) level in brain tissue and a significant increase ($p<0.05$) in MDA , glucose ,total cholesterol(TC) ,triacyglyserol (TAG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) in the serum and a significant decrease ($p<0.05$) in high density lipoprotein (HDL) and protein concentration in the serum group which exposed to x-ray compare with the control group .

The microscopical examination was shown in the Cx group brain tissue characterized by necrosis and congestion compare with control group . In conclusion , the 100kilorad x-ray exposure in male rabbits showed increase in the oxidative stress markers and Ach esterase activity in brain tissue and serum ,pathological lesions in the brain tissue of male rabbits .

المستخلص

أن الهدف من الدراسة الحالية هو تقيم تأثير اشعة اكس (X RAY) على بعض المعايير الوظيفية والافات النسجية التي تسببها في نسيج دماغ ذكور الارانب. قسمت عشوائيا عشرون من ذكور الارانب الى مجموعتين (10 ارانب لكل مجموعة)مجموعة السيطرة (C) والمجموعة المعرضة الى (Kilorad100) (Cx) لمدة ثلاثة اشهر, وعند دراسة بعض المعايير الفسلجية لوحظ زيادة معنوية ($P<0.05$) في مستوى المالونديهايد (MDA) و نشاط انزيم الاستيلكولينستريز (AchE) مع انخفاض معنوي ($p<0.05$) في مستوى الكلوتاثايون (GSH) في نسيج الدماغ.كما لوحظ زيادة معنوية ($p<0.05$) في مستوى MDA والكلكوز و الكوليسترول الكلي (TC) والثلاثي اسيل كوليسترول(TAG) والشحوم البروتينية الواطئة (LDL) والواطئة جدا(VLDL) في المصل مع انخفاض معنوي ($P<0.05$) في مستوى الشحوم البروتينية العالية (HDL) وتركيز البروتين الكلي في مصل كل من المجموعة المعرضة للاشعة بالمقاربة مع مجموعة السيطرة. اوضح الفحص المجهري لنسيج الدماغ للمجموعة المعرضة للاشعاع وجود تنخر مع احتقان مقارنة مع مجموعة السيطرة.

نستنتج من الدراسة الحالية ان تعرض ذكور الارانب لـ(100kilorad) من اشعة اكس يسبب زيادة في دلائل الاجهاد التأكسدي وزيادة في نشاط انزيم اسيتيل كولين استريز في نسيج الدماغ والمصل مع افات نسجية في الدماغ .

Introduction

Living organisms are continually exposed to ionizing radiation in nature as well as from nuclear weapons testing, occupations, consumer products, and medical procedures. The radiation from all of these sources together is called natural background radiation and is estimated to measure 180 to 200 mrem/person/y(1,2). Exposure to Uranium, Radium and Thorium, which occur naturally, are normally isolated geologically from the environment under shale and quartz. Man is responsible for disturbing them, digging them up and contaminating the environment.(3, 4)

Some early medical and dental users of x-rays, largely unaware of the hazards involved, accumulated considerable doses of radiation. As early as the year 1910, there were reports of cancer deaths among physicians, presumably attributable to x-ray exposure.(5) Skin cancer was a notable finding among these early practitioners; dentists. Ionizing radiation is a form of radiation with

sufficient energy to remove electrons from their atomic or molecular orbital shells in the tissues they penetrate (6,7). These ionizations, received insufficient quantities over a period of time, can result in tissue damage and disruption of cellular function at the molecular level. Of particular interest is their effect on deoxyribonucleic acids (DNA).(8)

High doses of ionizing radiation can lead to various effects, such as skin burns, hair loss, birth defects ,illness, cancer, and death. Within the same individual, a wide variation in susceptibility to radiation damage exists among different types of cells and tissues. Most sensitive are the white blood cells called lymphocytes, followed by immature red blood cells(9,10). Epithelial cells, high sensitivity; in terms of injury from large doses of whole-body external radiation, the epithelial cells which line the gastrointestinal tract are often of particular importance. Cells of low sensitivity include muscle and nerve, which are highly differentiated and do not divide(11). Delayed somatic effects of ionizing radiation result from somatic mutations and accumulated damage, and include impaired circulation, necrosis, fibrosis of skin and muscle tissue, loss of hair, loss of taste, impaired bone growth, susceptibility to disease, immunodeficiency, aplastic anemia, cataracts, and increased incidence of cancer.(12)

Humans are continually exposed to radiation from terrestrial sources. The risk of developing cardiovascular disease in patients exposed to radiation for diagnostic and therapeutic purposes, and from occupational radiation exposure remains a health concern. Cancer induction is the most important somatic late effect of low-dose radiation exposure. (2)

Leukemia is one of the most frequently observed radiation-induced cancers.(1,5) It accounts for one sixth of the mortality associated with radio carcinogenesis (13), with equal numbers of cancers of the lung, breast, and gastrointestinal tract. Thyroid cancer is also a concern for low-level exposure and late radiation effects, possibly accounting for 6% to 12% of the mortality attributed to radiation induced cancers.(1). Breast cancer is the major concern for women exposed to low-level radiation because of its high incidence in the unexposed population.5. Cancers of the stomach, colon, liver, pancreas, salivary glands, and kidneys are also induced by radiation (14)

The risk of radiation-induced bone, lung,(15) and skin cancers(16)is higher than other systemic cancers. Because radium is a bone-seeking element with a half-life of 1,600 years incidence of bone sarcomas (17). Acute CNS risks include altered cognitive function, reduced motor function, and behavioral changes, all of which may affect performance and human health. Late CNS risks are possible neurological disorders such as Alzheimer's disease, dementia, or premature aging (1).

The main objective of the present study was to evaluate the effect of chronic effects of ionizing radiation in the brain tissue of male rabbits by estimation of some physiological parameters in brain tissue and in the plasma and histopathological study of brain .

Material and methods

Experimental Protocol. A total of 20 three-month-old male rabbits(`1000–1500 g) were collected from animal house of Bagdad university .. After four weeks of acclimatization, the rabbits were randomly divided into two groups with ten animals in each group, control group (C) and treated group Cx (exposure to X –ray a 100kiolorad for three months) . Ninety days after x-ray exposure , overnight fasting, 5ml Blood samples were collected by cardiac puncture in plane tubes from all rabbits. The blood sample tubes were centrifuged at 3000 rpm for 2-5 minutes then serum separated to be stored at (-20ºC) to determine GSH , MDA and Acetyl cholin esterase AChE activity in the serum and brain total cholesterol (TC) ,glucose and protein in the serum .

Determination of AChE activity:- After getting the blood, rabbits have been killed and remove brain quickly and placed in containers that contain cool saline to remove blood and then the organs either are fixed in containers that contain 10% formaldehyde solution to prepare tissue sections. or quickly placed in containers that contain ice- cool saline to extract enzyme(brain tissue obtained for Ach esterase activity estimation and lipid per oxidation and histological sections) (18)**.**

Extraction Acetylcholinesterase method(19). The brain of male rabbit (5g) were homogenized in a glass Teflon homogenizer, containing 30ml of homogenization buffer (solution.2), in cool bath for

15 min and then centrifuged at 2,000 × g for 20 min at 4 ºC.. In this work, AChE activity in the rabbit brain, was determined colorimetrically by(20).

Preparation of extract and determination of lipid peroxidation , brain removed and (0.5 g) was homogenized in ice-cold physiological phosphate buffer, lipid peroxidation was determined colorimetrically by The absorbance of the supernatant obtained was read at 532 nm against the reagent blank.

Brain and serum glutathione (GSH) :- All analytical methods such as, photometric enzymatic, flourometric and HPLC methods, that used to determine tissues homogenate, erythrocyte and serum glutathione (GSH) depend on the action of sulfhydryl groups.5, 5 Dithiobis (2-nitrobenzoic acid) (DTNB) is a disulfide chromogen that is ready reduced by sulfhydryl group of (GSH) to an intensity yellow compound . The absorbance of the reduced chromogen is measured at 412 and is directly proportional to the GSH concentration.(22). Serum Protein concentration determination was performed as follows(24), serum total cholesterol and Triacylglycerol concentration estimation and high density lipoprotein (HDL-C) ,low density lipoprotein (LDL-C) and very density lipoprotein VLDL-C estimation as follows (25).

Statistical analysis

All the experiments were performed induplicate measurements and the results are shown as the mean ± S.E.Statistical analysis of data was performed with repeated measures analysis of variance (ANOVA) and $P \leq 0.05$ were considered to be significant also use LSD and SPSS statistical program (26)

Results and discussion

The tables (1) illustrated the mean values of MDA (1.26±0.003)µg/dl in the serum treated rabbits are significant increased ($p<0.05$) when compared to the control group(0.39±0.02) µg/dl while the same table cleared the significant decrease ($p<0.05$) and protein concentration (2.11±0.33)g/dl in serum GSH compared with control group .

The current study showed a significant increase ($p<0.05$) in mean values of TC , TAG, LDL and VLDL observed in x-ray exposure group when compared to the control table (1), this result agreement with (27) which showed that from 20 days after total body irradiation , a progressive increase in total serum cholesterol was seen. Low-density lipoprotein cholesterol progressively increased to a peak value of 82 ± 8 mg/dl at 80 days compared with 13 ± 3 mg/dl in un irradiated rats. There was also a transient increase in triglyceride levels 40 days after total body irradiation , which then declined to values present in un irradiated rats by 100 days . A single exposure to 15–60 Gy exerts an adverse long-term effect on cardiovascular function in the rat, resulting in morphological degeneration (28), mechanical dysfunction (29), damage to the endothelium and increased mortality (28), It has been suggested that radiation-induced cardiac injury is mediated by micro-vascular injury caused by inflammation and oxidative stress(30)but further studies are needed. Free radicals are generated during long-term exposure to extremely low levels of radiation, which will exhaust our defenses unless our body has an abundant reserve of antioxidants(27). free radicals" are formed and may play an important role in both cancer and aging. There is some evidence that these radicals are generated continually at a low rate as a byproduct of certain normal biochemical reactions in living cells, and that radiation simply accelerates their formation (31).

Table (1) effect of x-ray exposure on some serum physiological parameters on male rabbits

Parameter / Groups	MDA µ/dl	GSH µ/dl	TC Mg/dl	TAG Mg/dl	HDL Mg/dl	LDL Mg/dl	VLDL Mg/dl	Glucose mg/dl	Protein g/dl
C	0.39 ±.002	20.36 ±.61	99.51 ±2.68	87.53 ±1.35	32.67 ±.62	66.35 ±.56	24.68 ±.81	98.31 ±2.11	5.47 ±.60
Cx	1.26* ±.003	6.38* ±.44	233.24* ±6.84	191.24* ±1.71	14.07* ±.39	167.91* ±.45	40.02* ±.57	201.22* 6.95	2.11* ±.33

Mean ±SE n=10 (*)denote significant to differences under $p \leq 0.05$

C=control group , Cx = x-ray exposure for three months

Table (1)also illustrated the significant increase (P≤0.05) in mean value of glucose (201.31±6.95) mg/dl in treated group compare with the control group، Pancreatic beta cells are sensitive to reactive oxygen species (ROS) attack when they are exposed to oxidative stress, because of the relatively low expression of antioxidant enzymes such as catalase and glutathione peroxidase. Diabetes is typically accompanied by increased production of free radicals and/or impaired antioxidant defense capabilities, indicating a central contribution of reactive oxygen species. It is also a fact that ROS is one of the major factors that induce oxidative modification of DNA and gene mutation (27).

Table (2) effect of x-ray exposure on MDA and GH and Ach esterase on brain tissue in male rabbits

Parameter / Groups	MDA µ/dl	GSH µ/dl	Ach esterase uI/mg
C	0.36 ±0.072	18.15 ±0.059	0.58 ±0.02
Cx	0. 98* ±0.065	6.82* ±0.041	3.84* ±0.01

Mean ± SE * denote to deference under p ≤0.05

C=control group , Cx =x-ray exposure for three months group

Table (2) showed significant (P≤0.05) increase in MDA level of brain tissue (0.98±0.065)µ/dl compare with control group (0.36±0.072), while the same table illustrated the significant decrease in GSH concentration in group which is exposed 100kilorad x-ray for three months 6.82±0.041)compared with control group (18.15±0.059).

group Cx have shown a significant (P≤0.05) increase in mean value of Membrane -bound enzyme acetylcholinesterase specific activity level compared to control group , increase TC and TAG , LDL and VLDL in the serum showed an increase in cholesterol levels and Brain acetylcholinesterase (AChE) activity ,also previous studies showed that the increase in brain lipid peroxidation and decrease in antioxidant enzyme activities increased AChE activity (32).

The study of the effects of radiation on the nervous system particularly difficult because of the system own morphological and functional complexity , the response of the nervous system of man and on the effects of radation upon it are scanty and come mainly from survivors of nuclear bombings at Hiroshima and Nagasaki , patients irradiated for medical reasons ,people occupationally exposed and people irradiated accidently(33) . Ionizing radiation damages biological tissues by exciting or ionizing their atoms and molecules. Depending on the radiation dose and the biochemical processes altered, damage may be prompt (34,35) The ionization events and free radicals produced by radiation cause damage to vital cellular components. Cell death during mitosis (mitotic death) is generally caused by unrepaired or improperly repaired chromosomal damage. Cell death may also occur by apoptosis (6. 36).

The histological sections in figures (2) and(3) showed that there were necrosis , fibrosis and congestion compared with control , this result aggrement with(37),this is probably a reflection of reduced aerobic metabolism of the brain tissue. Astocytes may become hypertophic within three weeks or longer after doses of 100 rads or morethis being a reflection of altered vascular permeability to proteins, oligodendroglial cells associated with the myelination process ,undergo acute swelling or hypertrophy also a wide dose range (38,39).

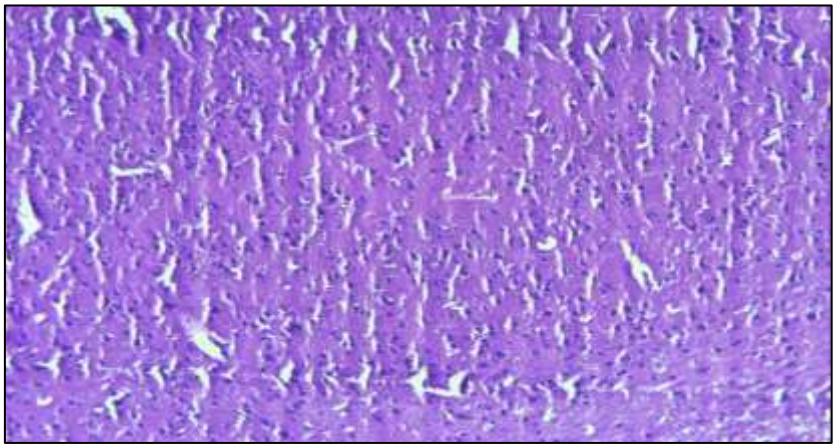

Figure (1) histological section of brain tissue in control group (H&E10X)

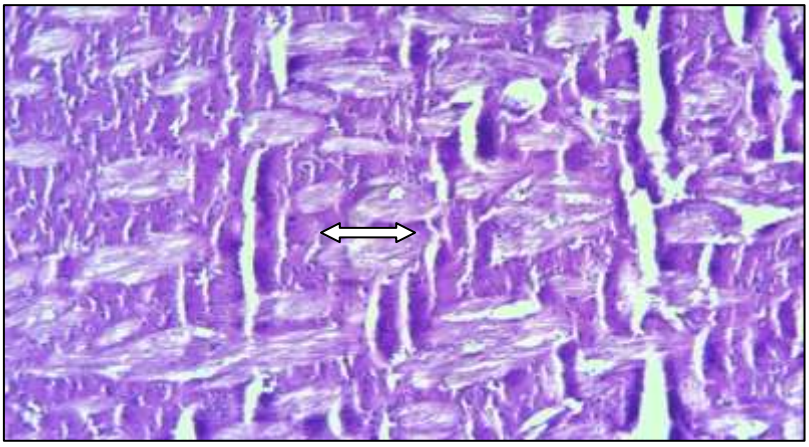

Figure (2) histological section of brain tissue in treated group show necrosis ⟺)(H&E10X)

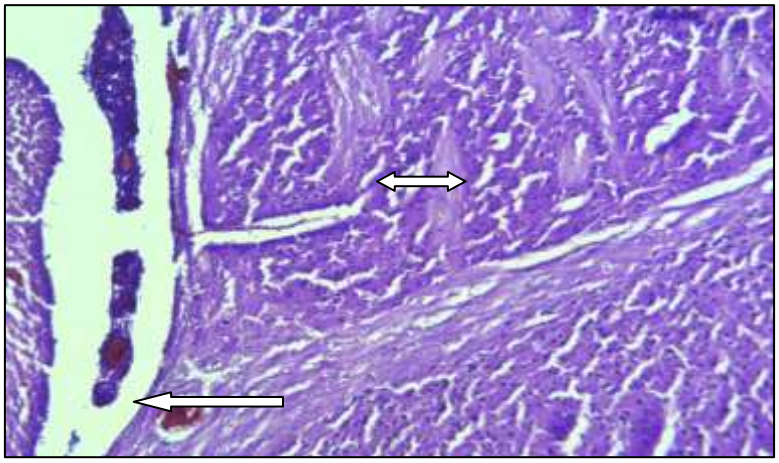

Figure (3) histological section of brain tissue in treated group show necrosis

⟺) and congestion (⟸).(H&E10X)

Reference

1- **Hall ,E.J ;Giaccia ,A.(2008).** Radiobiology for the Radiologist. 6th ed. Philadelphia, PA: Lippincott, Williams & Wilkins.
2- **Nias, A.H.(1998).** An Introduction to Radiobiology. 2nd ed. New York, NY: Wiley.
3- **Lieble ,S. A. and Sheline ,G.E.(1987)** Radiation therapy for neoplasm of the brain .J.Neurosurg 66:1-22.
4- **Matsuam,M. (1987).**Radiation damage to the brain ,no to shinkei brai and nerve.39:696
5- **United Nations Scientific Committee on the Effects of Atomic Radiation to the General Assembly (UNSCEAR)(2000).** Sources and Effects of Ionizing Radiation, A Report of the, Volume II: Effects, New York.
6- **Adams, M.J. Lipshultz, S.E; Schwartz, C; Fajardo, L.F; Coen Vand Constine LS.(2003).** Radiation-associated cardiovascular disease: manifestations and management. Semin Radiat Oncol. 13:346–356.
7- **Baker, J.E; Fish, B. J; Haworth, S. Strande;J. Komorowski, R; Migrino, R. Doppalapudi, A. Harmann; L. X; Hopewell, J; Moulder, J.(2009).** Total body irradiation increases risk of coronary sclerosis, degeneration of heart structure and function in a rat model. Int J Radiat Biol. 85:1089–1100.
8- **Bryan ,N.S. Fernandez, B.O; Bauer, S.M; Garcia-Saura, M.F; Milsom ,A.B. Rassaf, T;Maloney, R.E; Bharti, A; Rodriguez, J; Feelisch M.(2005).** Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. Nat Chem Biol.1:290–297.
9- **Alexandra, C. M ; Merriline, SShilpa. K. and Thomas.(2010.).**late and low –level effect of ionizing radiation Consequences of Radiological and Nuclear Weapons.
10- **Fajardo, L.F; Stewart ,J.R.(1970).** Experimental radiation-induced heart disease. I. Light microscopic studies. Am J Pathol. 59:299–316.
11- **Shukitt-Hale, B; Casadesus, G; Cantuti-Castelvetri, I; Rabin, B. Joseph, J. (2003)**.Cognitive deficits induced by56Fe radiation exposure. Adv. Space Res., 31(1):119–126
12- **Steel ,G.G.(2002).** Basic Clinical Radiobiology. 3rd ed. London, England: Arnold.
13- **Gardner ,M.J; Snee, M.P; Hall, A.J; Powell, C.A; Downes, S; Terrell J.D.(1990).** Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria. *BMJ*. 300:423–429.
14- **Buckley, P.G; Bines, W.P; Kemball, P.T; Williams, M.K.(1997**). Development of revised Ionizing Radiations Regulations. J Radiol Prot. 18(3):115–118.
15- **Stroian** ,**G,;Martens, C; Souhami, L; Collins, D.L; Seuntjens** ,**J. (2008).**Local correlation between monte-carlo dose and radiationinduced fibrosis in lung cancer patients. *Int J Radiat Oncol Biol Phys*. 70(3):921–930.
16- **Martin, M; Lefaix, J.L; Pinton, P; Crechet, F; Daburon, F.(1993).** Temporal modulation of TGF-beta 1 and beta-actin gene expressionin pig skin and muscular fibrosis after ionizing radiation. Radiat Res. 134(1):63–70.
17- **National Council on Radiation Protection and Measurements.(2009).**Ionizing Radiation Exposure of the Population of the United States.Bethesda,MD:NCRP; 2009. NCRP Report 160.
18- **Khandkar, M.A. ; Mlnherjee, E. ; Parmar, D.V; and Katyare, S.S.(1995).** Alloxan-diabetes alters kinetic properties of the membrane-bound form, but not of the soluble form, of acetylcholinesterase in rat brain, Biochem. J., 307, 647-649 .
19- **Rakonczay, Z.(2003).** Potencies and selectivities of inhibitors of acetylcholinesterase and its molecular forms in normal and alzheimer's disease brain. Acta Biologica Hungarica ,54 (2) 183–189.
20- **Ellman,G.L.;Courtney, K.D. ;Andres, V.J and Featherstone, R.M. (1961).**A new and rapid colorimetric determination of acetylcholinesterase activity,Biochem. Pharmacol. 7 :88– 95.
21- **Burtis ,C. and Ashwood, E. (1999).** Textbook of clinical chemistry. 3d Ed.London. 941.

22- **Bradford, M.(1976).** A rapid and sensitive method for the quantitation of Microgram quantities of protein utilizing the principle protein . Analytical Biochemistry. 72 – 248 – 254.
23- **Friedewald,W.;Levy,Y and Fredrickson(1972).** Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. Clin. Chem., 18:499-502.
24- **Dunean, R.C; Knapp, R.G; and Millerill, M.C. (1983).** Introductory biostatistics for the health sciences (2nd Ed). Wiley Medical publication.
25- **Baker, J.E. Fish, B J; Haworth, S. Strande, J; Komorowski, R; Migrino, R. Doppalapudi, A. Harmann, L; Li, X; Hopewell, J. Moulder; J. (2009).**Total body irradiation increases risk of coronary sclerosis, degeneration of heart structure and function in a rat model. Int J Radiat Biol. 85:1089–1100.
26- **Dalla, Y; Singh, N; Jaggi, A. S; Singh, D. and Ghulati, p.(2009).**Potential of ezetimibe in memory deficits associated with dementia of Alzheimer's type in mice. *Indian J Pharmacol*, 41(6): 262-267.
29. Kruse, J.J; Zurcher, C; Strootman, E.G; Bart, C.I; Schlagwein, N; Leer, J.W. Wondergem, J.(2001). Structural changes in the auricles of the rat heart after local ionizing irradiation. Radiother Oncol. 58:303–311.
30. Wondergem, J; van der Laarse, A; van Ravels, F.J; van Wermeskerken, A.M; Verhoeve, H.deGraaf, B.W; Leer, J.W.(1991) In vitro assessment of cardiac performance after irradiation using an isolated working rat heart preparation. Int J Radiat Biol. 59:1053–1068.
31. Ito K, Hirao, A; Arai, F. (2004). Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature. 431(7011):997–1002.
32. Ghareeb ,D .and Hussein, H.(2008). Vanadium improves brain acetylcholinesterase activity on early stage alloxan-diabetic rats. Neuroscience Letters ,436: 44–7.
33. Schull ,W.J.(1983). Late radiation responses in man: current evaluation from results from Hiroshima and Nagasaki. Adv Space Res. 3(8):231–239
34 –Lauk, S; Kiszel, Z. Buschmann, J; Trott, K.R.(1985). Radiation-induced heart disease in rats. Int J Radiat Oncol Biol Phys. 1985;11:801–808..
35. **Stewart, F.A; Hoving, S; Russell, N.S.(2010).** Vascular damage as an underlying mechanism of cardiac and cerebral toxicity in irradiated cancer patients. Radiat Res. 174:865–869.
36. **Schultz-Hector, S; Trott, K.R.(2007**)..Radiation-induced cardiovascular diseases: is the epidemiologic evidence compatible with the radiobiologic data? Int J Radiat Oncol Biol Phys. 67:10–18.
37. **Chodick, G; Bekiroglu, N. Hauptmann, M.(2008).** Risk of cataract after exposure to low doses of ionizing radiation: a 20-year prospective cohort study among US radiologic technologists. Am J Epidemiol. 168(6):622–637.
38. **Cucinotta, F.A; Schimmerling, W; Wilson, J.W; Peterson, LE; Badhwar G.D; Saganti, P.B; Dicello, J.F.(2001).** Space radiation cancer risks and uncertainties for Mars missions. Radiat Res. 156:682–688.
39. **Nobuya, M; Takashi, T; Kengo, M; Tomoshisa, F. and Taskashi ,O. (2003)** radiation damage to the normal monkey brain . experimental study induced by interstitial irradiation . Acta Med. Okayama vol 57.no.3pp123-131.