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Hemodynamic and oxidative stress effects of gamma-radiation in both male and female rats

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Abstract

Objective. The present study aimed to determine the alteration in hemodynamic and oxidative stress caused by gamma-irradiation in both sexes of rats. **Materials and Methods:** Twenty-four albino rats were randomly divided into two groups The first group includes twelve rats (Six males and six females) served as control, while the rats of the second group (six males and six females) were exposed to gamma radiation through 0.763 Gy from Cesium-137 radioactive source for two weeks. **Results**: Gamma radiation exposure significantly raised malondialdehyde (MDA) levels. Furthermore, serum AST activity was greatly altered by gamma-radiation, while the ALT activity significantly increased by the gamma radiation in female rats only. Irradiated animals caused signification in blood pressure. Gamma-irradiation animals for both sexes resulted in significant decrease in serum calcium (Ca^{+2}) levels in both male and female rats. **Conclusion**, our results demonstrated that gamma- irradiated rats could increase tissue injuries markers and lipid peroxidation productions. Interestingly, the elevated blood pressure by gamma-ray exposure may be returned to high free radicals and changes in calcium ions.

Introduction

The pathophysiological consequences of gamma ray are mediated by cellular, chemical and physical responses initiated after ionizing radiation [1]. The water is the main part of the cell, it is primarily the ionization of water which leads to formation of secondary species with short life time's such as the hydroxical radical [2]. However, DNA is the target which it's damaged could result in cellular death and genetic changes [3]. There is a considerable evidence in the literature that radiation damage, is to a large extent, caused by the overproduction of reactive oxygen species (ROS), including superoxide anion ($O2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2), that decrease the levels of antioxidants, resulting in oxidative stress and cellular damage.,[4], [5].

Studies indicated that low irradiation doses in the environmental exposures would raise the risk of heart disease [6]. However [7] demonstrated that radiation-induces endothelium dysfunction and increase the arterial blood pressure. Though, experiments on non-anesthetized and anesthetized rats, demonstrated that irradiation led to significant elevation in the level of arterial blood pressure without any changes in cardiac contractility[8]. It is important to note that irradiated animals exhibited distinct and sustained signs of hypertension,[9]on the other hand, radiation alters the form and function of the BK(Ca) channel which contribute to related vascular abnormalities,[10]. In addition, [11] suggested that BK(Ca) can operate as a crucial factor for radiation-induced arterial hypertension. The results suggest that irradiation-evoked inhibition of the BK(Ca) current in aortic vascular smooth muscle cell is mediated by phoshokinase C ,[12].

Calcium is the central regulator of excitation-contraction coupling, which controls muscle contraction during each heartbeat,[13]. Contraction of heart cell is initiated by a transient rise in intracellular calcium ion levels where small alteration in it, can form functional changes in cardiac output. This indicates thatelevation in heart calcium level by gamma exposure will cause the shortage in its function, [14]. According to previous studies, gamma irradiation has toxic effect on hepatic cells, which led to significant increase of histological abnormalities (Hepatic vein lesions and cellular necrosis) and liver injury[15], [16], [17]. Investigators have reported significant elevation in the activity of liver enzymes after gamma irradiation, serum ALT, AST and GGT levels significantly decreased with the different gamma radiation doses in rat blood serum when compared with the control, [18], [19], [20]. Because the variation of response to gamma radiation in males and females has not been studied yet. Besides, Kurdistan environment may have such sources of gamma radiation, which exposed for both human sexes. Therefore, the objective of the current experiment is to identify the difference between males and females rats irradiated to gamma ray regarding hemodynamic and oxidative stress variables.

Materials and method:

Irradiation

Whole body gamma-irradiation of animals was performed using the Cesium-137 radioactive source of 2.5 MBq activity which provide a uniform gamma-ray exposure of about 2.2 mR/h at a distance 10 cm from the source. The rats exposed to this gamma-ray exposure rate for 14 days and thus received an approximate dose of 0.743 Gy.

Animals and housing

Adult male and female albino rats (*Rattus norvegicus*) were used in this study. All rats were weighing about (240 - 280 gm.) and (7-9) weeks of age at the time when the experiment started. Animals were housed in plastic cages bedded with wooden chips. They were housed under standard laboratory conditions. The animals were given standard rat pellets and tap water ad libitum. The employed experimental animals were met the criteria of ethic rules of the supervising committee of College of Science

Experimental Design

This experiment was designed to study the effects of gamma exposure on some hemodynamic, liver function and serum electrolyte measurements. Twenty-four rats were randomly divided into four groups, each with six individuals and the treatments were continued for 2 weeks as the following: Group 1 and 2 (Control), the rats (males and females) were given standard rat chow and tap water ad libitum and the animals were not exposed to whole body gamma-radiation. Group 3 and 4: The rats (males and females) were given standard rat chow and the animals were whole body gamma irradiated with 0.743 Gy of gamma-ray .

Collection of blood samples

At the end of the experiment, the rats were anesthetized with ketamine hydrochloride (50 mg/kg). Blood samples were taken by cardiac puncture into chilled tubes and centrifuged at 3000 rpm for 20 minutes; then sera were stored at $-85C^0$ until assay.

Blood pressure and heart rate measurement

At the end of the experiment, systolic BP and heart rate the blood pressures of rats were measured by the tail-cuff method in all groups using a power Lab (ADInstruments, power lab 2/25). Rats were placed in a restraining chamber and warmed to an ambient temperature of about $37C^0$ typically taking about 15 minutes after that occluding cuffs and pneumatic pulse transducers were placed on the rats' tails. Five readings were taken for each rat, the highest and lowest and any associated with excess noise or animal movement were discarded. The equipment was calibrated every time before using by comparison with a mercury column sphygmomanometer.

Biochemical determination

Determination of serum sodium, potassium and chloride ion concentrations

Serum Na^{+}, K^{+},Cl^{-},ionizedcalcium (iCa^{++}),total calcium (nCa^{++}) concentrations and serum pH were determined using fully automated electrolyte analyzer, ELITE,USA .

Determination of serum malondialdehyde (MDA)

The level of serum MDA was determined spectrophotometrically with a TBA solution. In brief to 150µl serum sample added the followings: 1ml trichloroacetic acid 17.5 %, 1ml of 0.66 % thiobarbituric acid (TBA), mixed well by vortex, incubate it in boiling water for 15 minutes, & then allowed to cool. Then add 1ml of 70 % TCA, & let the mixture to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 45 minutes, & take out the supernatant for scanning spectrophotometrically .

Determination of serum AST and ALT

Serum AST and ALT were determined by colorimetric method kit (BIOLABO . SA, France).

Statistical analysis

Results are expressed as means ± SEM and statistical analysis was performed by statistical software (SPSS version 15). The comparisons between groups were done by independent student T-test. The bar charts were made by Graph Pad Prism (Version 5). Values were considered to be significantly different when $P < 0.05$.

Results

Experiments were undertaken 24 rats in both sexes, except for control animals, they exposed to gamma irradiation for 14 days and then some physiological parameters were measured. As shown in Figure 1, MDA as indicator of the oxidative stress, and lipid peroxidation, exhibited very high significant increase in radiation exposure (3.256±0.430) , (5.280±0.450) as compared with control groups (0.892±0.357), (0.750±0.260) in both male and female groups ,respectively. Furthermore, serum AST activity was greatly altered by gamma-radiation exposure in both male and female rats as compared to controls (Figure 2). On other hand, the ALT activity significantly increased (66.500± 5.698) the gamma radiation in female rats as compared with the control group (53.580± 4.9586) (Figure 3), while it did not significantly change in male rats.

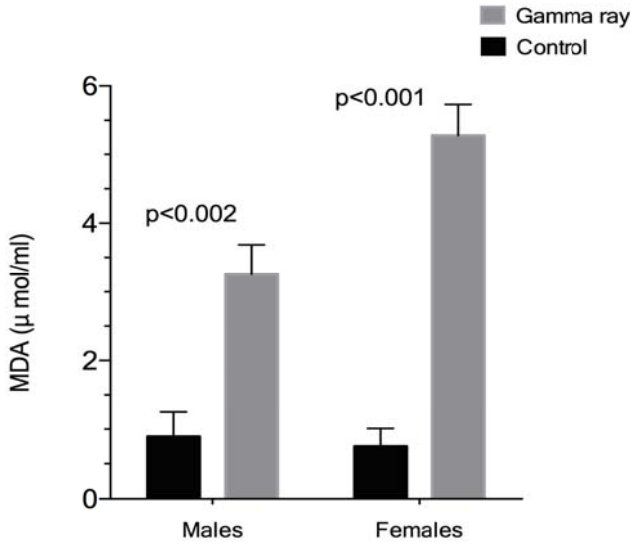

Figure1: Effects of gamma-ray on serum MDA levels in both sexes of rats

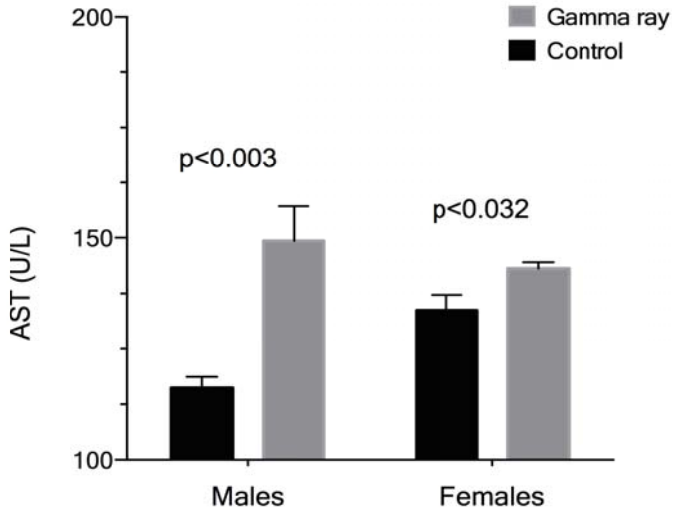

Figure2: Effects of gamma-ray on serum AST activity in both sexes of rats

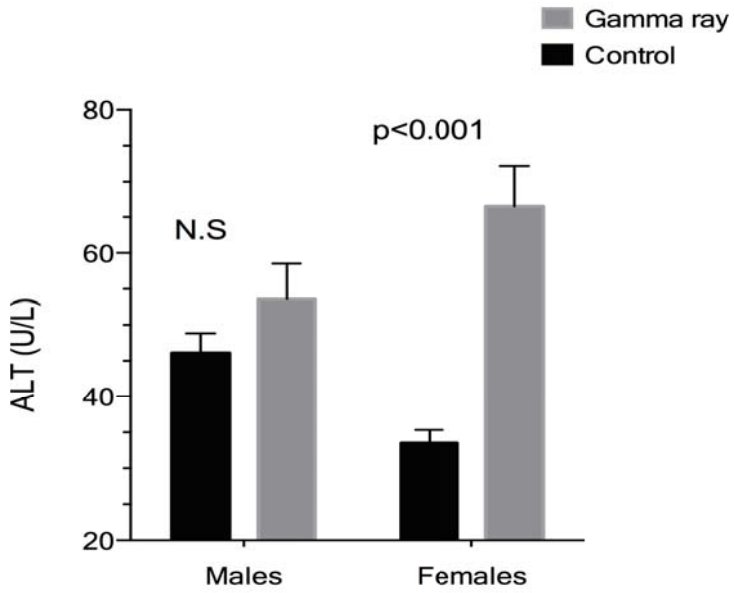

Figure3: Effects of gamma-ray on serum ALT activity in both sexes of rats

Statistical analysis revealed that SBP in high significant was elevated in male rats (135.600± 3.0659 mmHg) after two weeks of gamma-radiations as compared with control group (117.800± 1.777 mmHg) Also in female irradiated rats, SBP was increased but in low significant from 123.800± 0.9618mmHg to 116.800± 1.777mmHg in control rats (Figure 4). However, H.R did not change significantly by this exposure. Table 1 shows the impact of gamma-irradiation on the levels of serum Na^{+}, K^{+},Cl^{-},iCa^{++} ,nCa^{++} ion concentrations and serum pH . Gamma-irradiation animals for both sexes only resulted in significant decrease in serum iCa^{+2} and nCa^{++} levels in both male and female rats, while non significant differs were observed in serum Na , K^{+}, Cl^{-} concentrations and serum pH in both male and female groups.

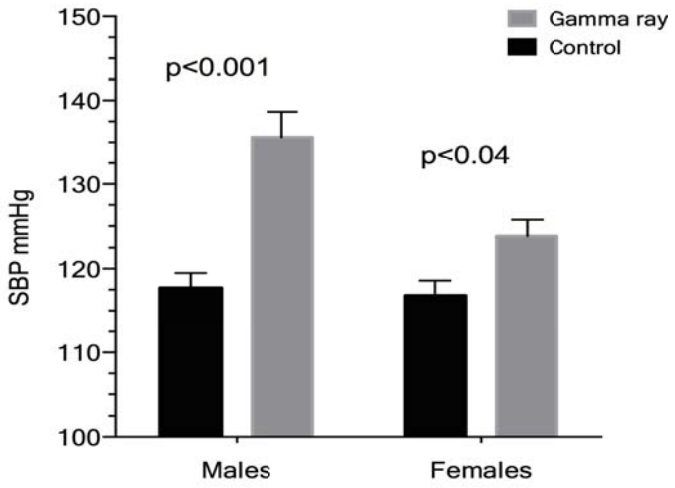

Figure 4: Effects of gamma-ray on systolic blood pressure (SBP) in both sexes of rats

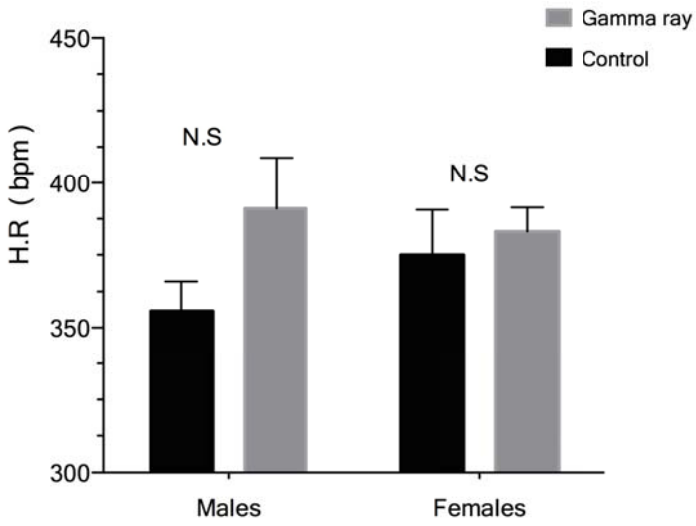

Figure 5: Effects of gamma-ray on heart rate (H.R) in both sexes of rats

Table 1 Effects of gamma ray on serum electrolytes in both sexes of albino rats. (Data expressed as Mean ± MSE)

Sexes	Males		Females	
Electrolytes / Groups	Control	Gamma ray	Control	Gamma ray
Serum Na^{+} mmol/L	138.8±3.4099	136.6±2.3617	139.0± 10.13	144.0± 4.875
Serum K^{+} mmol/L	5.000± 0.3066	5.0333± 0.1725	4.840±0.2138	4.960± 0.312
Serum Cl^{-} mmol/L	107.3± 1.085	113.3± 2.1396	110.0± 6.255	115.8 ± 4.142
Serum iCa^{++} mg/dL	4.683± 0.289	3.4500± 0.1765 **	4.563± 0.109	3.700± 0.206 **
Serum pH	7.915± 0.050 2	7.821 ± 0.0738	7.756± 0.036	7.796± 0.025
Serum nCa^{++} mg/dL	5.750 ± 0.0764	4.350± 0.296 **	4.560± 0.258	4.390± 0.259 *

* P<0.05 **P<0.01

Discussion

The present study proposed that gamma- ray (Cesium-137) irradiated rats either males or females evokes free radical production with causing tissue injuries by elevating their makers like AST and ALT enzymes activities, besideselevating blood pressure. Gamma radiation exposure for two weeks in both male and female rats significantly raised oxidative stress marker, measured as MDA level. Several previous studies also showed that animals when exposed to cesium-137 irradiation caused lipid peroxidation and increases MDA levels, [3], [21], [22] and concomitant the level of antioxidant level rises, [23], [24], [7]. However, a study revealed that gamma-radiation which induced oxidative stress it also decreases antioxidant enzyme activity, [25]. As obtained from the results, the level of lipid peroxidation was more elevated in females than the male rats (Figure,1), this difference may be due to that female body fats produced more oxidative stress in term of lipid peroxidation than males with low fat body mass. [26]. Although, it is well established that estrogens and progesterone have antioxidant properties, [27], [28] but their antioxidant activity did not override high fat mass composition in females which may highly exposed to peroxidation after animal irradiation.

The current results shown in Figure 2 and 3, indicated that irradiated female rats for two weeks significantly elevated serum AST and ALT activities, while serum AST was increased in high significant in irradiated male rats, whereas serum ALT activity did not record significant change. [3]revealed that cesium-137 irradiated rats showed a significant increase in serum AST levels. Also [29] observed in their results that gamma-irradiation markedly increases serum AST and ALT activities in rats[30] ,[28]. The precise mechanisms involved in tissue injuries by cesium-137 irradiated rats have not been fully elucidated. Accordingly, the elevation of lipid peroxidation, as MDA by gamma radiation might explain such injuries and changes in AST and ALT enzyme markers. [31].

As seen in Figure 3, serum ALT did not change significantly after gamma-irradiation in male rats. The reason behind this result is not precisely known, however the possible hypothesis for explaining this effects is that testosterone may protect liver injuries, [32] who showed that testosterone may act in part via an effect on the key regulatory lipogenic enzymes to protect liver injuries. Steroid hormone deficiencies may be somewhat have roles in protecting free radical formation. One might also argue that, since MDA level was highly raised in females rather than males,the data presented here may suggest that female liver may be more sensitive to gamma-radiation than males due to low levels of testosterone hormones.

In the present study, statistical analysis revealed that irradiated animals for two weeks showed highly significant elevation in blood pressure in males, while low significant blood pressure elevation was detected in females. There was evidence that radiation induces endothelium dysfunction, [33].Although damage in endothelium releases vasoconstrictors like endothelin-1, [34]. On the other hand, this elevation of blood pressure did not returned to change in heart rate as obtained from the present result and shown in Figure 5. Also [8] demonstrated that irradiation caused to rises in blood pressure without changes in cardiac contractility.

Furthermore, irradiated animals may alter BK ca^{+2} channels which strongly related in vascular malfunction and arterial hypertension development, [11] and recently [10] showed that irradiation–evoked inhibition of the BK Ca^{+2} current in aortic vascular smooth muscle cells which mediated by phoshokinase C. Interestingly, serum calcium was significantly reduced after gamma-irradiated male and female rats, (Table 1). Changes in Ca^{+2} concentration stimulate BK ca^{+2} channels, [35]. Hence, the explanation of increased blood pressure in irradiated rats would be through changes in BK ca^{+2} channels and consequently changes blood pressure. According to the present results, irradiated animals didn't change serum electrolytes, Na^+ ,K^+ and Cl^-, so it can be concluded that elevated blood pressure may not be associated with electrolyte changes. Another possible mechanism for blood pressure elevation related to gamma radiation may due to increasing of free radicals, as[36] showed that free radicals constrict vascular smooth muscles.

In conclusion, our results demonstrated that gamma-ray(cesium-137) irradiated rats could increase tissue injury and lipid peroxidation production markers. Interestingly, the elevated blood pressure by gamma-ray

exposure was accompanied with elevation in free radicals and calcium ions. However, tissue injuries in both male and female rats were observed by radiation, but the results suggested that female rats liver may be more sensitive to injuries than male rats.

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References

[1] Soule BP, Hyodo F, Matsumoto K, Simone NL, Cook JA, Krishna MC, Mitchell JB, "*The chemistry and biology of nitroxide compounds*", Free radical biology & medicinem 42(11), pp1632-1650,(2007).

[2] Kim J, Park JW, Park KM, "*Increased superoxide formation induced by irradiation preconditioning triggers kidney resistance to ischemia-reperfusion injury in mice*", American journal of physiology Renal physiology, Vol. 296 No.5, pp 1202-1211(2009).

[3] Gillard N, Goffinont S, Bure C, Davidkova M, Maurizot JC, Cadene M, Spotheim-Maurizot M, "*Radiation-induced oxidative damage to the DNA-binding domain of the lactose repressor*",The Biochemical journal 2007, 403(3):463-472.

[4] Han J, Won EJ, Lee BY, Hwang UK, Kim IC, Yim JH, Leung KM, Lee YS, Lee JS, "*Gamma rays induce DNA damage and oxidative stress associated with impaired growth and reproduction in the copepod Tigriopus japonicus"*, Aquatic toxicology (Amsterdam, Netherlands), Vol. 152, pp 264-272 (2014).

[5] Spitz DR, Azzam EI, Li JJ, Gius D, "*Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: a unifying concept in stress response biology*", Cancer metastasis reviews, Vol. 23 No.3-4, pp 311-322 (2004).

[6] Little MP, Tawn EJ, Tzoulaki I, Wakeford R, Hildebrandt G, Paris F, Tapio S, Elliott P, "*Review and meta-analysis of epidemiological associations between low/moderate doses of ionizing radiation and circulatory disease risks, and their possible mechanisms*", Radiation and environmental biophysics, Vol. 49 No.2, pp 139-153(2010).

[7] Dynlacht JR, Tyree C, Valluri S, DesRosiers C, Caperell-Grant A, Mendonca MS, Timmerman R, Bigs by RM, *"Effect of estrogen on radiation-induced cataractogenesis*", Radiation research, Vol.165,No.1, pp 9-15 (2006).

[8] Tishkin SM, Rekalov VV, Ivanova IV, MoreLand RS, Soloviev AI, "*Ionizing non-fatal whole-body irradiation inhibits Ca2+-dependent K+ channels in endothelial cells of rat coronary artery: possible contribution to depression of endothelium-dependent vascular relaxation*. International journal of radiation biology, Vol.83, No.3, pp 161-169 (2007).

[9] Soloviov AI, Tyshkin SM, Khromov OS, Stefanov OV, "*Change in contractile vascular function in arterial hypertension of different genesis and its correction with phosphatidylcholine liposomes*", Fiziolohichnyi zhurnal (Kiev, Ukraine : 1994), Vol.48, No.6, pp 11-18 (2002).

[10] Kyrychenko S, Tishkin S, Dosenko V, Ivanova I, Novokhatska T, Soloviev A, "*The BK(Ca) channels deficiency as a possible reason for radiation-induced vascular hypercontractility*". Vascular pharmacology, Vol.56 No. 3-4, pp142-149 (2012).

[11] Darenskaia NG, Korotkevich AO, Maliutina TS, Nasonova TA, "*Quantitative patterns in the clinical manifestations of radiation sickness in large laboratory animals exposed to radiation at superlethal doses. Changes in the regulation of cardiovascular system function"*, Radiatsionnaia biologiia, radioecologiia / Rossiiskaia akademiia nauk, Vol.37, No.3, pp 431-437 (1997).

[12] Kizub IV, Pavlova OO, Ivanova IV, Soloviev AI, *"Protein kinase C-dependent inhibition of BK(Ca) current in rat aorta smooth muscle cells following gamma-irradiation"*, International journal of radiation biology, Vol.86,No.4, pp 291-299 (2010).

[13] Bohm J, Leshinsky-Silver E, Vassilopoulos S, Le Gras S, Lerman-Sagie T, Ginzberg M, Jost B, Lev D, Laporte J, "*Samaritan myopathy, an ultimately benign congenital myopathy, is caused by a RYR1 mutation",* Acta neuropathologica, Vol.124,No.4, pp 575-581 (2012).

[14] Sun YB, Irving M, "*The molecular basis of the steep force-calcium relation in heart muscle*", Journal of molecular and cellular cardiology, Vol.48, No.5, pp 859-865(2010).

[15] Moriconi F, Malik I, Ahmad G, Dudas J, Rave-Frank M, Vorwerk H, Hille A, Hess CF, Ramadori G, Christiansen H, "*Effect of irradiation on gene expression of rat liver adhesion molecules: in vivo and in vitro studies. Strahlentherapie und Onkologie*", Organ der Deutschen Rontgengesellschaft [et al], Vol.185, No.7, pp 460-468 (2009).

[16] Chetyrkin SV, Chernukhina LA, Porokhniak LA, Donchenko GV, Kuz'menko IV, Klimenko EP, "*Effect of low doses of radiation on vitamin A and E levels in rat liver*", Ukrainskii biokhimicheskii zhurnal, Vol.71,No.2, pp 38-42 (1999).

[17] Nikitchenko Iu V, Roman'ko M, Dziuba VM, Fuks PP, "*Lipid peroxidation and regulation of it in rat blood and liver under the experimental alimentary radionuclide effect*", Ukrainskii biokhimicheskii zhurnal, Vl.73, No.5, pp 43-48 (2001).

[18] Mansour HH, Ismael Nel S, Hafez HF, *"Ameliorative effect of septilin, an ayurvedic preparation against gamma-irradiation-induced oxidative stress and tissue injury in rats.* Indian journal of biochemistry & biophysics, Vol.51, No.2, pp 135-141 (2014).

[19] Rave-Frank M, Malik IA, Christiansen H, Naz N, Sultan S, Amanzada A, Blaschke M, Cameron S, Ahmad S, Hess CF et al, "*Rat model of fractionated (2 Gy/day) 60 Gy irradiation of the liver: long-term effects*", Radiation and environmental biophysics, Vol.52, No.3, pp 321-338 (2013).

[20] Lee JW, Byun EH, Sung NY, Raghavendran HR, Byun EB, Kim JH, Choi JI, Shin MG, Byun MW, *"Effect of gamma irradiation on the efficacy of beta-glucan against acetaminophen induced toxicity in mice*", Chemico-biological interactions, Vol.180, No.1, pp 98-105 (2009).

[21] Gil'iano N, Konevega LV, Noskin LA, "*Immediate and delayed effects of low doses of beta-radiation in mammalian cells in culture*", Radiatsionnaia biologiia, radioecologiia / Rossiiskaia akademiia nauk Vol.51, No.6, pp 670-676 (2011).

[22] El-Missiry MA, Fayed TA, El-Sawy MR, El-Sayed AA, "*Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury",* Ecotoxicology and environmental safety Vol.66, No.2, pp 278-286 (2007).

[23] Kordysh EA, Goldsmith JR, Quastel MR, Poljak S, Merkin L, Cohen R, Gorodischer R, "*Health effects in a casual sample of immigrants to Israel from areas contaminated by the Chernobyl explosion*", Environmental health perspectives, Vol. 103, No.10, pp 936-941 (1995).

[24] Racine R, Grandcolas L, Blanchardon E, Gourmelon P, Veyssiere G, Souidi M, *"Hepatic cholesterol metabolism following a chronic ingestion of cesium-137 starting at fetal stage in rats"*, Journal of radiation research, Vol.51, No.1, pp 37-45 (2010).

[25] Won EJ, Lee JS, *"Gamma radiation induces growth retardation, impaired egg production, and oxidative stress in the marine copepod Paracyclopina nana".* Aquatic toxicology (Amsterdam, Netherlands), Vol.150, pp 17-26 (2014).

[26] Wang C, Wang X, Tian H, Fang F, Han X, *"Association of abdominal fat distribution by computed tomography with body mass index and metabolic syndrome in Chinese elders*", Zhonghua yi xue za zhi Vol.94, No.12, pp 908-912 (2014).

[27] Toillon RA, Magne N, Laios I, Castadot P, Kinnaert E, Van Houtte P, Desmedt C, Leclercq G, Lacroix M, "*Estrogens decrease gamma-ray-induced senescence and maintain cell cycle progression in breast cancer cells independently of p53",* International journal of radiation oncology, biology, physics, Vol.67, No.4, pp 1187-1200 (2007).

[28] Adaramoye OA, Okiti OO, Farombi EO, *"Dried fruit extract from Xylopia aethiopica (Annonaceae) protects Wistar albino rats from adverse effects of whole body radiation. Experimental and toxicologic pathology",* official journal of the Gesellschaft fur Toxikologische Pathologie, Vol.63, No.7-8, pp 635-

643 (2011).

[29] Pradeep K, Ko KC, Choi MH, Kang JA, Chung YJ, Park SH, "*Protective effect of hesperidin, a citrus flavanoglycone, against gamma-radiation-induced tissue damage in Sprague-Dawley rats*", Journal of medicinal food, Vol.15, No.5, pp 419-427 (2012).

[30] Ramadan LA, Roushdy HM, Abu Senna GM, Amin NE, El-Deshw OA, "*Radioprotective effect of silymarin against radiation induced hepatotoxicity",* Pharmacological research : the official journal of the Italian Pharmacological Society, Vol.45, No.6, pp 447-454 (2002).

[31] Kanazawa I, Tanaka K, Sugimoto T, "*DPP-4 inhibitors improve liver dysfunction in type 2 diabetes mellitus".* Medical science monitor: international medical journal of experimental and clinical research Vol. 20, pp 1662-1667 (2014).

[32] Kelly DM, Nettleship JE, Akhtar S, Muraleedharan V, Sellers DJ, Brooke JC, McLaren DS, Channer KS, Jones TH, "*Testosterone suppresses the expression of regulatory enzymes of fatty acid synthesis and protects against hepatic steatosis in cholesterol-fed androgen deficient mice*", Life sciences, Vol. 109 No.2, pp 95-103 (2014).

[33] Hu S, Chen Y, Li L, Chen J, Wu B, Zhou X, Zhi G, Li Q, Wang R, Duan H et al. "*Effects of adenovirus -mediated delivery of the human hepatocyte growth factor gene in experimental radiation-induced heart disease*", International journal of radiation oncology, biology, physics, Vol.75 No.5, pp 1537-1544 (2009).

[34] Laczik R, Soltesz P, Szodoray P, Szekanecz Z, Kerekes G, Paragh G, Rajnavolgyi E, Abel G, Szegedi G, Bodolay E, "*Impaired endothelial function in patients with undifferentiated connective tissue disease: a follow-up study*", Rheumatology (Oxford, England), Vol.53 No.11, pp 2035-2043(2014).

[35] Ko EA, Han J, Jung ID, Park WS, "*Physiological roles of K+ channels in vascular smooth muscle cells*", Journal of smooth muscle research = Nihon Heikatsukin Gakkai kikanshi, Vol. 44 No.2, pp 65-81 (2008).

[36] Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT, "*Melatonin membrane receptors in peripheral tissues: distribution and functions*", Molecular and cellular endocrinology, Vol. 351 No.2, pp 152-166 (2012).