Impacts on the third line of defense specialized against microbial infection as a result of exposure to gamma-radiation

Mohamed Anwar Kassem Abd El-Halim[1*] and Sherifa Mostafa Mohamed Sabra[2]
[1]Biomedical Physics Specialty, Physics and Astronomy Dept., Science College, King Saud University, KSA
[2]Microbiology specialty, Microbiology Br., Biology Dept., Science College, Taif University, KSA

Abstract: To understand the toxicity and the potential role of gamma-radiation (GR) as a therapeutic tool, the effects of different GR doses on haematological and dimensional properties of rats' blood were investigated *in vivo.* 60 healthy male Wistar-Kyoto rats were used in this study, and were randomly divided into 5 groups, 4GR rat groups (1st group was radiated with 5GR, 2nd group 25Gy; 3rd group with 50Gy, 4th group with 100Gy and 5th group was the control). Different haematological and dimensional parameters were measured using the standard haematological technique for complete blood count (CBC). A significant decrease in white blood cells (WBCs) count and lymphocytes (LYM) was observed compared with the control. While a significant increase in monocytes (MON), neutrophils (NEU), basophils (BAS), and eosinophils (EOS) were observed. A non-significant decrease in platelets (PLTs) count was observed with GR compared with the control**.**

Keywords: Gamma-radiation (GR), In-vivo, CBC, WBCs, LYM, MON, NEU, BAS, EOS, PLTs.

INTRODUCTION

The leucocytes (WBCs), are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All WBCs are produced and derived from a multi-potent cell in the bone marrow known as a haematopoietic stem cell (Maton *et al*., 1997). The number of WBCs in the blood is an indicator of the disease. The WBCs count is usually between (4-11x10^9/L blood). In the US this is usually expressed as (4.000-11.000/ microliter blood) (Vital and Health, 2005).

The changes in blood cell counts are still considered the most sensitive biological evidences for excessive acute exposure to both external and internal radiation. The CBC gives important information about kinds and numbers of cells in the blood, especially WBCs and PLTs that helps in diagnosing conditions, as infection, and many other disorders. The radiation can penetrate the living cells and deposit within them in random fashion, leading to radiation damage (Jacob and Jagetia, 1992).

The risk of transfusion-associated graft-versus-host disease (TA-GVHD) is related to the number of viable T cells transfused. Whether WBC-reduced blood components would carry a decreased risk of TA-GVHD was considered, and the allogeneic mixed LYM reaction was used as an *in vitro* model for TA-GVHD. An exponential decline in the mixed LYM reaction was found as a result of either an arithmetic increase in the dose of GR to responding cells or a logarithmic decrease in the number of un-radiated responding cells. GR of responding cells with 600cGy or a 0.6 log10 reduction in the number of responding cells produced a 95% decline in the mixed LYM reaction. It was relative risk of TA-GVHD resulting from the use of standard cellular components (Dzik and Jones, 1993) and its biological effects on living systems at the molecular, cellular and neuro-endocrine (Daga *et al*., 1995).

In vitro properties, PLTs were examined in four series of radiated and controls PLTs, each obtained from the same 15donors. Radiation with 3000cGy was performed on days 0, 3 and 5. Comparable *in vitro* properties were measured in the radiated and control PLTs, whether radiation was performed on day 3 or day 5. GR has no adverse effect on PLTs quality in extremely WBCs-reduced (Robert *et al.,* 2001).

The effects of low-dose GR on DNA damage, chromosomal aberration, and DNA gene expressions in whole blood and peripheral LYM became evident. At absorbed doses of 5 and 10cGy, significant increases in DNA strand breaks and oxidative base damage observed, which determined as formamidopyrimidine-DNA-glycosylase (FPG)-sensitive sites. However, GR at doses up to 500cGy did not significantly increase the level of 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxodG), which determined by HPLC with electrochemical detection (HPLC-ECD). GR as low as 5cGy, caused chromosomal aberrations, which determined as dicentric and deletion frequencies. Significant genotoxic effects of GR can be observed even at a low dose of 5cGy. Furthermore, at 20cGy, GR induced a significant decrease in the mRNA expression of both hOGG1 and XRCC1 repair genes, which determined by the reverse RT-PCR. The expression levels of hOGG1 and XRCC1 mRNA were inversely

**Corresponding author:* e-mail: abdelhalimmak@yahoo.com

correlated with the levels of FPG-sensitive sites and DNA strand breaks. The finding of decreased expression levels for hOGG1 and XRCC1 in GR LYM has not been reported elsewhere. The observations of Wanwisa *et al.* (2006) have suggested that the geno-toxic effects of GR may be due to a combination of DNA-damaging effects and reduced DNA repair capacity. It was administered various doses for seven consecutive days before exposing them to a single dose of 4.56Gy 60Co-GR on whole body. The SI treatments accelerated the recovery of circulating WBCs and reticulocytes (RETs) seven days following GR. These effects were dose-dependent, and the strongest effect on most biomarkers was seen with an intermediate dose (Li-Hua *et al.,* 2006).

Five different and diverse types of WBCs exist; these types are distinguished by their physical and functional characteristics. MON and NEU are phagocytic (LaFleur-Brooks, 2008). WBCs and PLTs suspended in the blood plasma. In addition, blood is crucial for removing carbon dioxide, lactic acid, urea, and etc. from the same cells and tissues. In addition, blood is responsible for immune system, clotting, regulation of body temperature, and many other functions. Its value not only decreases but also when more liquid enters into the intravasale spaces (hydration). In contrary, the increase in its value is observed when the organism loses liquids, so decreasing the plasma volume (dehydration due to diarrhea, burning of a large surface of the body that obtained due to exposure to GR). The blood viscosity increases rapidly with an increase in the haematocrit (Karsheva *et al*., 2009). In medicine, there is limited knowledge on the haematological indices, alterations induced by different GR doses. Thus, this study was aimed to follow-up the effects of GR on the rats by measuring the total and differential WBCs and PLTs counts for GR rats blood compared with the control *in vivo*.

MATERIALS AND METHODS

Animals and treated groups

A total of 60 healthy male Wistar-Kyoto rats were used in this study. Animals were randomly divided into five groups. Four radiated rat groups (n=20) and one control group (n=5), were exposed to GR as follows: (1st group was radiated with 5Gy, 2nd group was radiated with 25Gy, 3rd group was radiated with 50Gy, 4th group was radiated with 100Gy, and 5th control group. The rats were anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. Blood sample of nearly 2ml collected into EDTA-polypropylene tubes for whole blood. All experiments conducted in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee.

Haematological measurements

WBCs and PLTs were measured using the standard haematological techniques. Haematological Auto-Analyzer (Orphee Mythic 22 Haematological Analyzer; Diamond Diagnostic; USA) was used to determine different haematological parameters, which were WBCs, NEU%, LYM%, MON%, EOS%, BAS%, PDW%, and PLTs.

STATISTICAL ANALYSIS

The results of this study were expressed as mean ± standard error (Mean ± SE). To assess the significance of the differences between the control group and the four GR rat groups, a statistical analysis was performed using one-way analysis of variance (ANOVA) for repeated measurements, with the significance assessed at 5% confidence level.

RESULTS

Table 1 and fig. 1 shows the mean prevalence of total and differential of WBCs counts after GR, the relationship between WBCs count and different GR were done for rat blood; the means were significantly different ($p<0.05$)). Total WBCs were decreased with GR (5, 25, 50 and 100Gy) as (7.000, 1.200, 1.200 and 1.200/microliter) than the normal control (8.200/microliter).

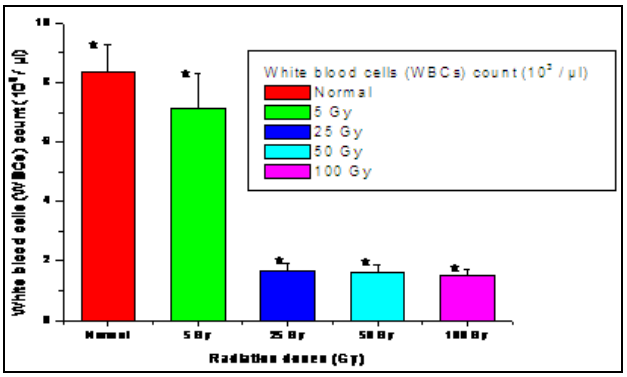

Fig. 1: The relationship between WBCs count and different GR were done for rat blood [*means that the means are significantly different ($p<0.05$)]

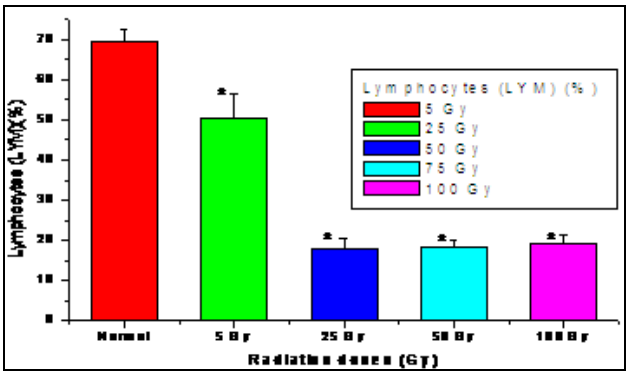

Fig. 2: The relationship between LYM and different GR were done for rat blood [*means that the means are significantly different ($p<0.05$)]

Table 1: The mean prevalence of total and differential of *WBCs counts after GR

*GR Doses	*C.	5Gy	25Gy	50Gy	100Gy
*WBCs/microliter					
	8,200	7,000	1,200		
Differential %					
*LYM	69%	49%	16%		
*MON	6%	5.5%	11%	16%	16.5%
*NEU	17%	22%	52%	45%	37%
*EOS	1.5%	3%	6.5%	5.5%	12%
*BAS	5%	9.7%	12.5%	14%	13%

*GR: Gamma radiation, *C.: Control, *WBCS: White blood cells corpuscles, *LYM: Lymphocyte,*MON: Monocyte, *NEU: Neutrophil, *EOS: Eosinophil, *BAS: Basophil

Table 2: PLTs counts calculated at different GR doses

Blood Indices	*C.	5Gy	25Gy	50Gy	100Gy
Platelet distribution width (PDW%)	17.17±1.37	16.34±2.95	17.68±2.91	16.12±1.81	14.29±1.33
Platelets count (PLT)	780.17±32.96	626±49.00	723.08±101.83	806.67±37.15	698.6±24.69

*C.: Control; All results are represented as Mean ± SE [*means that the means are significantly different (p<0.05)]

Table 1 and fig. 2 shows the mean prevalence of total and differential of WBCs counts after GR, the relationship between LYM and different GR were done for rat blood, the means were significantly different (p<0.05). LYM deceased with GR (5, 25, 50 and 100Gy), as (49, 16, 16 and 16%) than the normal control by 69%.

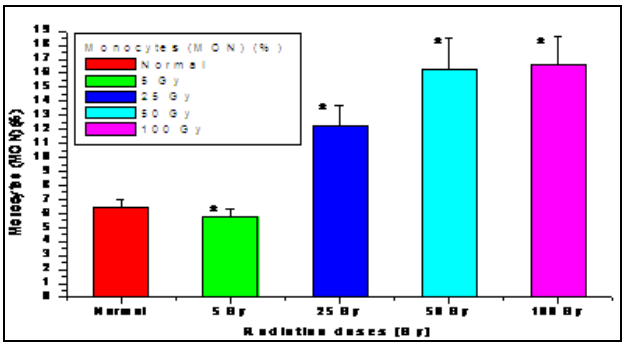

Fig. 3: The relationship between MON and different GR were done for rat blood [*means that the means were significantly different (p<0.05)]

Table 1 and fig. 3 shows the mean prevalence of total and deferential of WBCs after GR, the relationship between MON and different GR were done for rat blood [the means are significantly different (p<0.05)]. MON deceased with GR 5Gy as 5.5% and then increased as (11, 16 and 16.5%) with GR in (25, 50 and 100Gy) than the control by 6%.

Table 1 and fig. 4 show the mean prevalence of total and deferential of WBCs counts after GR, the relationship between NEU and different GR made for rat blood [the means are significantly different (p<0.05)]. NEU increased with GR (5, 25, 50 and 100Gy) as (22, 52, 45 and 37%) than the normal control by 17%.

Table 1 and fig. 5 shows the mean prevalence of total and deferential of WBCs counts after GR, the relationship between EOS and different GR made for rat blood [the means are significantly different (p<0.05)]. EOS increased with GR (5, 25, 50 and 100Gy) as (3, 6.5, 5.5 and 12%) than the normal control by 1.5%.

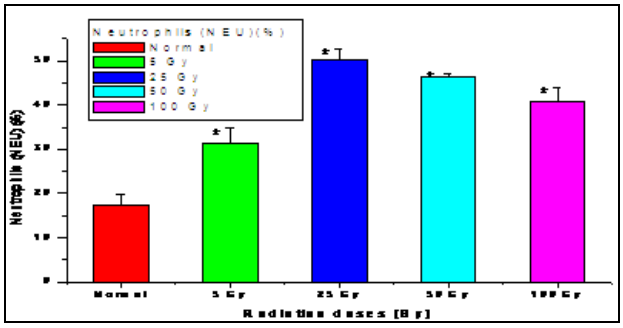

Fig. 4: The relationship between NEU and different gamma radiation doses for rat blood [*means that the means were significantly different (p<0.05)]

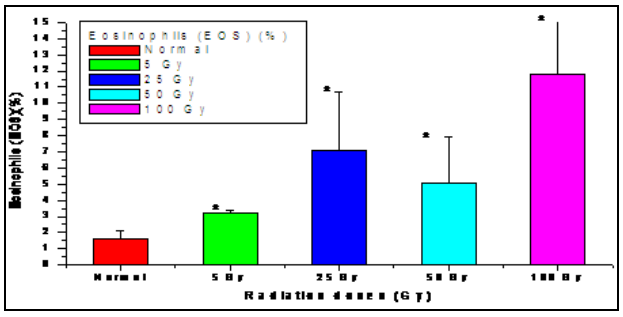

Fig. 5: The relationship between EOS and different GR were done for rat blood [*means that the means were significantly different (p<0.05)]

Table 1 and fig. 6 show the mean prevalence of total and deferential of WBCs counts after GR, the relationship between BAS and different GR made for rat blood [the means are significantly different ($p<0.05$)]. EOS increased with GR (5, 25, 50 and 100Gy) as (9.7, 12.5, 14 and 13%) than the control by 5%.

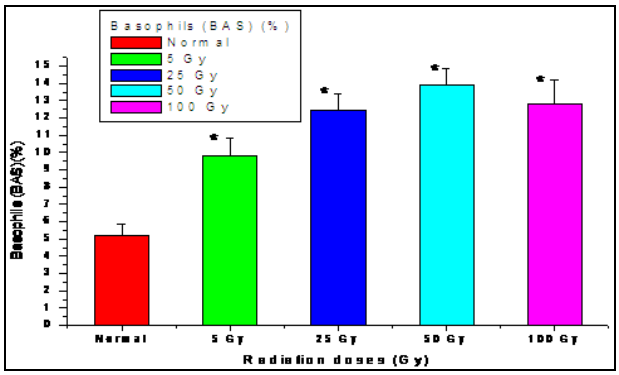

Fig. 6: The relationship between BAS and different gamma radiation were done for rat blood [* means that the means were significantly different ($p<0.05$)]

Table 2 shows PLTs counts compared with different GR doses, decrease in PDW was observed**.** The PLTs count decreased with 5, 25, 50 and 100Gy GR compared with the control.

DISCUSSION

The results of this study clarify the decrease in total WBCs and LYM%, while an increase in granulocytes (NEU, EOS and BAS); MON were undulating in decrease and increase.

Low PLT count (thrombocytopenia) may be the cause of prolonged bleeding or other medical conditions. The changes in WBCs and PLTs will be the enhancing factors for inflammatory microbial infection, toxic reactions, bone marrow problem and disturbance in the function of immune system efficiency. The changes in blood cell counts are still considered the most sensitive biological evidences for excessive acute exposure to both external and internal radiation. CBC gives important information about the kinds and numbers of cells in the blood, especially WBCs and PLTs and helps in diagnosing conditions, as infection, and many other disorders. It became apparent that, the radiation can penetrate the living cells and deposit within them in random fashion, leading to radiation damage (Jacob and Jagetia, 1992).

An exponential decline in the mixed LYM reaction was found as a result of either an arithmetic increase in the dose of GR to responding cells or a logarithmic decrease in the number of un-radiated responding cells. GR of responding cells with 600cGy or a 0.6 log10 reduction in the number of responding cells produced a 95% decline in the mixed LYM reaction. It was relative risk of TA-GVHD resulting from the use of standard cellular components (Dzik and Jones, 1993). Radiated and control PLTs, whether radiation was performed on day 3 or day 5. GR had no adverse effect on PLTs quality in extremely WBCs-reduced (Robert *et al.,* 2001). Exposure to low-dose GR is common in certain occupations, but the biological and health effects from such exposure remain to be determined. The effects of low-dose GR on DNA damage, chromosomal aberration and DNA repair gene expressions in whole blood and peripheral LYM.

The observations suggest that the geno-toxic effects of GR may be due to a combination of DNA-damaging effects and reduced DNA repair capacity (Wanwisa *et al*., 2006).

Blood is responsible for immune system, clotting, regulation of body temperature, valuable information about the changes in blood plasma and many other functions. Its value decrease not only but also when more liquid enters in the intra-vassal space (hydration). In contrary, the increase in its value is observed when the organism loses liquids, so decreasing the plasma volume (dehydration due to diarrhea, burning of a large surface of the body that obtained due to exposure to GR) and the blood viscosity increases rapidly with the increase in haematocrit (Karsheva *et al*., 2009).

CONCLUSIONS

This study showed that WBCs count and LYM% significantly decreased after GR compared with the control**.** The decrease in WBCs count might indicate a disturbance in the immune system function, therefore it becomes susceptible to any microbial infection**.** A low WBCs count may point toward bone marrow problems or related to some medications, such as radiotherapy. A significant increase in the majority of MON%, NEU%, EOS% and BAS% were observed after GR espouse compared with the control**.** PLTs count decreased with the different GR compared with the control**.** A low PLTs count might be the cause of prolonged bleeding or other medical conditions. This study suggests that the patients always expose to GR, are suffering from a decrease in WBCs and PLTs, as well as the medical advice to allow dis-connect exposure to GR. The buffy coat transfusion, which contains WBCs and PLTs, also enhancing production of WBCs and PLTs by good and complete meals which contain minerals, vitamins and principle substances to build-up a new healthy WBCs and PLTs.

ACKNOWLEDGEMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the research Group Project No.RGP-VPP-285.

REFERENCES

Daga SS, Jain VK and Goyal PK (1995). Radio response of leucocytes in peripheral blood of mice against gamma-radiation and their protection by *Liv*. *Probe* **52,** 222-226.

Dzik W and Jones K (1993). The effects of gamma irradiation versus white cell reduction on the mixed lymphocyte reaction. *Transfusion*, **33**(6): 493-496.

Jacob PS and Jagetia GC (1992). Vinblastine treatment increases the clastogenic effects of radiation in mouse bone marrow. *Mutation Research*, **280**: 287.

Karsheva M, Dinkova P, Pentchev I and Ivanova T (2009). Blood rheology a key for blood circulation in human body. *J. Chem. Technol. Metall ,* **44**(1): 50-54.

LaFleur-Brooks M (2008). Exploring medical language: A student-directed approach (7th ed.). St. Louis, Missouri, US: Mosby Elsevier. p.398.

Li-Hua S, Hong-Li Y and Dong-Lian C (2006). Protective effects of soybean isoflavone against gamma-irradiation induced damages in mice. *J. Radiat. Res.,* **47**: 157-165.

Maton D, Hopkins J, McLaughlin Ch W, Johnson S, Warner MQ, LaHart D, Wright JD and Kulkarni DV (1997). Human Biology and Health. Englewood Cliffs, New Jersey, US: Prentice Hall. ISBN 0-13-981176-1. OCLC 32308337, 1993.

Robert Z, Stefan S, Jürgen Z, Anke G, Volker W, Andreas R and Reinhold E (2001). Effect of gamma radiation on the *in vitro* agreeability of WBC-reduced apheresis platelets. *Transfusion,* **41**(2): 236-242.

Vital and Health Statistics Series 11, No. 247 (03/2005).

Wanwisa S, Panida N and Mathuros R (2006). Effects of low-dose gamma radiation on DNA damage, chromosomal aberration and expression of repair genes in human blood cells. *Int. J. Hygiene and Environmental Health,* **209**(6): 503-511.