Volume 17, No 1 | International Journal of Radiation Research, January 2019

An in-vivo study on the energy dependence of X-ray biological effectiveness

M.A. Fadel[1], R.H. Bakr[2*], A.R. El-Sersy[2]

[1]Department of Biophysics, Faculty of Science, Cairo University, Giza, Egypt
[2]Ionizing Radiation Metrology Lab, National Institute for Standards, Tersa St. El-Haram, Giza, Egypt

► ***Original article***

Corresponding authors:
Dr. R.H. Bakr,
Fax: *+202 338 67451*
E-mail:
reham.hamdy87@gmail.com

Revised: February 2018
Accepted: June 2018

Int. J. Radiat. Res., January 2019; 17(1): 111-118

DOI: 10.18869/acadpub.ijrr.17.1.111

ABSTRACT

Background: The International Commission on Radiological Protection (ICRP) has attributed the same relative risk for X and gamma radiations of all energies. Several studies have proven that the biological effect of low energy photon is more than that of higher ones. The assessment of risks is important due to the wide use of low energy X-rays for mammography screening and other diagnostic applications. ***Materials and Methods***: Five X-ray beam qualities characteristics according to ISO -4037 and consultative committee of ionizing radiation (CCRI) were studied in details. In pilot study made to investigate the minimum dose that cause measurable biological effects, one hundred male albino rats were equally divided into five groups namely A, B, C, D and E. Group A was used as control while animals of other groups were whole body exposed to different radiation doses from 137Cs γ-rays. For studying the biological effect energy dependency, eighty male albino rats were equally divided into 8 groups namely F, G, H, I, J, K, L and M. Group F was used as control and was not exposed to any type of radiation while animals of other groups were whole body exposed to the same dose of radiation but at different dose rates and different energies. Blood samples were collected and serum samples were separated for further biochemical investigations. Biochemical investigations for blood sera included alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), blood urea nitrogen (BUN), creatinine (CREA), calcium (Ca++) and Creatine phosphokinase (CPK) level. ***Results:*** The results showed remarkable variation of the measured biochemical parameters levels with different photon energies that reflect the energy dependency of the investigated parameters. ***Conclusion:*** It's clear from the data that the same absorbed dose delivered to the exposed animals induces different effect according to the used photon energy. Low energy X-rays were found to be more biologically effective than higher ones.

Keywords: *X-ray beam qualities, Biological effect, Energy dependent.*

INTRODUCTION

Several efforts were made to determine the relative biological effectiveness (RBE) in terms of different types of cell damage and its dependence on photon energy. These studies showed that low energy photons are more effective in inducing cell damage than high energy photons [1, 2]. ICRP 60 [3-5] has assigned a radiation weighting factor of 1 for X-rays, gamma rays and electrons of all energies.

Data from a range of laboratories around the world, with few exceptions, show ultra soft X-rays to have increased effectiveness for a wide range of biological end-points compared to equal doses of conventional X-rays or gamma rays [6-8], with RBE values typically increasing with decreasing ultra soft X-ray energy. Induction of DNA double-strand breaks (DSBs) by low-LET radiations reflects clustered damage produced predominantly by low-energy [9], secondary electron "track ends". Relative to ^{60}Co gamma

rays, the relative biological effectiveness (RBE) for cell inactivation at 10% survival and for induction of DSBs increases as the photon energy of the ultrasoft X-rays decreases. Therefore, ultrasoft X-rays are more efficient per unit dose than gamma radiation at inducing DNA DSBs [10].

Consequently one may wonder if the radiation weighting factor is valid for all photon energies. Therefore, the aim of the present work is to accurately determine the photon energy and radiation doses and study the biological effect energy dependency of whole-body exposure of rats to a definite dose with different energies of X and γ rays.

MATERIALS AND METHODS

Irradiation facility

Male albino rats were irradiated to γ-rays from ^{60}Co (Gammatron S80) with original activity of 178.5 TBq at June 1990 made by Siemens, ^{137}Cs source manufactured by atomic energy of Canada Ltd in ○April 1970 of model GB150 serial No. 37 and original activity of 1000 Curies and to X-rays from Philips MCN-323 double pole in Ionizing Radiation Metrology Laboratory (IRML) at National Institute for Standards (NIS), Egypt. The temperature and pressure were taken before and after irradiation by calibrated thermometer and barometer.

X and γ ray dosimetry

The absorbed dose was measured using a secondary standard system (PTW UNIDOS, 10001-10522) and ionization chamber. This system is traceable to the SI unit (Gy) through primary dosimetery standard of the Bureau International des Poids et Measures (BIPM) (France (2017)). ^{60}Co and ^{137}Cs doses were measured using 1000 cc PTW ion chamber. The setting up for the X-ray tube is constructed according to the TRS No. 469 [11] of IAEA as shown in figure (1).

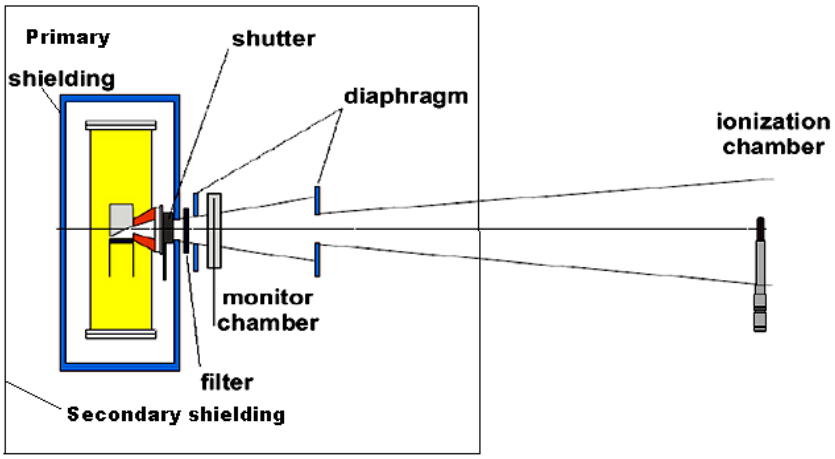


Figure 1. X-ray setting as in TRS 469.

Animals

Male albino rats of about 8 weeks age and mean weight of (≈150 g ±10%) were used in this study. The animals were under similar environmental conditions of temperature, illumination and acoustic noise. The animals were kept in special cages that allow normal ventilation and daylight. Cleaning and changing water and food were done twice per day and received the same diet during the experiment. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Institutional ethical approval number was: CUIS 39-16 CU-IACUC.

All animals of all groups were whole body exposed to radiation. Exposure was performed using specially designed cage with specific dimensions to assure accurate and homogenous

radiation dose delivered by animals. To minimize the uncertainty of the irradiation dose, each animal was individually exposed and the cages used in exposure were with dimension nearly as that of animals.

X-ray beam characterization

Characterization of X-ray beam qualities according to ISO 4037 and consultative committee for ionizing radiation (CCRI) was performed [12, 13]. ISO-filters of Cu and AL with purity of 99.9% were used for added filtration and Half Value Layer (HVL) measurements to achieve the precise beam qualities using the method proposed by TRS-457 [14].

Choice of exposure dose

In pilot study to investigate the minimum dose that cause measurable biological effect, one hundred male albino rats were equally divided into five groups namely A, B, C, D and E. Animal of group A were used as control group and didn't receive any treatment. Groups B, C, D and E were exposed to 20, 50, 100 and 150 mGy respectively of ^{137}Cs γ-rays in one shoot at dose rate of 51.58 mGy/min. Blood samples were collected from an eye vein in plastic tubes and were allowed to clot at room temperature then centrifuged at 3000 rpm for 15 minutes. Serum samples were separated for biochemical analysis. Biochemical parameters measured were alanine aminotransferase (SGPT) and aspartate aminotransferase (SGOT) as hepatic functions, blood urea nitrogen (BUN) and creatinine (CREA) as renal functions in addition to ionized calcium (Ca^{++}) and Creatine phosphokinase (CPK) as heart enzyme.

Energy dependence of biological effects

- For studying the energy dependence of biological effect, eighty Male albino rats were equally divided into 8 groups namely F, G, H, I, J, K, L and M.
- Animals of group F were used as control and didn't receive any treatment while animals of group G, H, I, J, K, L and M were exposed to the same radiation dose but at different dose rates and energies.
- At low dose rate (1.66 mGy/min), animals of groups G, H and I were exposed to 25 KV X-ray, ^{137}Cs gamma rays and ^{60}Co gamma rays respectively.

At high dose rate (51.58 mGy/min), animals of groups J, K, L, M were exposed to 50, 100, 135 and 180 KV X-ray respectively.

After radiation exposure, blood samples were collected and serum samples were separated for further biochemical analysis.

Biochemical sera analysis were accomplished at the Auto-analyzer Unit, National Research Center (NRC) Cairo- Egypt using Olympus Chemical Auto-analyzer, Model, Au400; GmbH, Wendenstr. 14-18, D-20097 Hamburg, Germany.

Statistical analysis

Statistical analysis of the data was performed by calculating arithmetic means and standard deviations. All of these measurements were performed for all animal groups and the mean reading was used to calculate the mean and standard deviation for each.

RESULTS

X-ray beam characterization

Before using any X-ray machine, the beam quality should be determined. The beam quality of the X-ray is governed not only by kVp and mA but also by half value layer (HVL) in standard reference material such as Cu or Al, homogeneity coefficient (h) and the effective energy.

Table 1shows the beam qualities used for medium and low energy X-ray. The X-ray mean energy was obtained from the corresponding HVL as the method proposed by El-Sersy *et al.* [15] from the equation (1):

$$E_{(keV)} = 70.38\ (HVL_{mmCu})^{0.326} \quad (1)$$

Table 2shows the mean energy of the studied beam qualities with corresponding kVp.

From table 1, one can notice that, the first HVL for the studied X-ray beam qualities were in agreement with that of CCRI at which the used ion chambers was calibrated [16].

The effective energy of the used beam qualities was calculated using equitation 1 and represented in table .2

Table 1. X-ray beam qualities of NIS for medium and low energy.

Tube kVp (V)	Needed added filter (mm)		1st HVL	1st HVL Of CCRI	2nd HVL	Homogeneity coefficient (%)	Type of chamber used for dosimetry
	Al	Cu					
25	------	------	0.35Al	0.24 Al	0.57Al	89.47	PTW23342
50	1.025	------	2.25Al	2.262Al	2.91Al	80.37	PTW 30013
100	1.010	------	0.15Cu	0.148 Cu	0. 37 Cu	45.34	PTW 30013
135	------	0.199	0.49Cu	0.494 Cu	0.85Cu	57.13	PTW 30013
180	-------	0.4	0.99Cu	0.994 Cu	1.58Cu	61.18	PTW 30013

Table 2. Effective energy of the studied qualities of the beam.

Tube kVp (V)	Mean X-ray energy (keV)
25	18
50	29.5
100	42
135	60
180	82

Choice of exposure dose

The average values of the measured SGPT, SGOT, BUN, Ca^{++}, CPK and CREA ± standard deviation for all exposed groups (A to E) to different doses from Cs-137 in the pilot study are given in table 3. The data shows significant decreases in the SGPT, SGOT, BUN and Ca^{++} and increase in CPK and CREA enzymes for exposed animals to different doses as compared with the control.

Energy dependence of biological effects

The average values of SGPT, SGOT, BUN, CPK, CREA and Ca^{++} ± standard deviation for all irradiated groups (F to M) to a definite dose with different photon energies are represent in histograms for both low and high dose rates and shown in figure 2.

Data of figure 2 illustrated that the biological effect is energy dependent where the hazard increase with energy decrease. To confirm the conclusion above, the relative numerical values of SGPT, BUN and CPK, for the exposed animals to the control was plotted with energy and represented in figure 3.

Table 3. Shows the average and standard deviation levels of SGPT, SGOT, BUN, Creatinine, Ca++ and CPK in blood serum of animals from the different groups as compared with control.

Direct effect						
Group ID	SGPT U/l	GOT U/l	BUN mg/dl	CREA mg/dl	Ca^{++} mg/dl	CPK U/l
A	48.0±3	155.0±5	38.00±2.0	0.29±0.03	7.00±0.3	24.67±0.58
B	43.5±9	123.5±2***	20.90±1.0***	0.32±0.03	7.50±0.3	359.5±2.00***
C	42.6±1	136.8±2**	22.21±0.2***	0.45±0.02***	5.85±0.7**	165.0±9.00***
D	31.0±3**	132.0±6**	21.73±2.0***	0.50±0.03***	5.35±0.7**	135.0±7.00***
E	26.0±3***	137.0±6**	16.39±2.0***	0.45±0.03***	5.05±0.6***	124.0±9.00***

*means significant, ** means highly significant, *** highly highly significant.

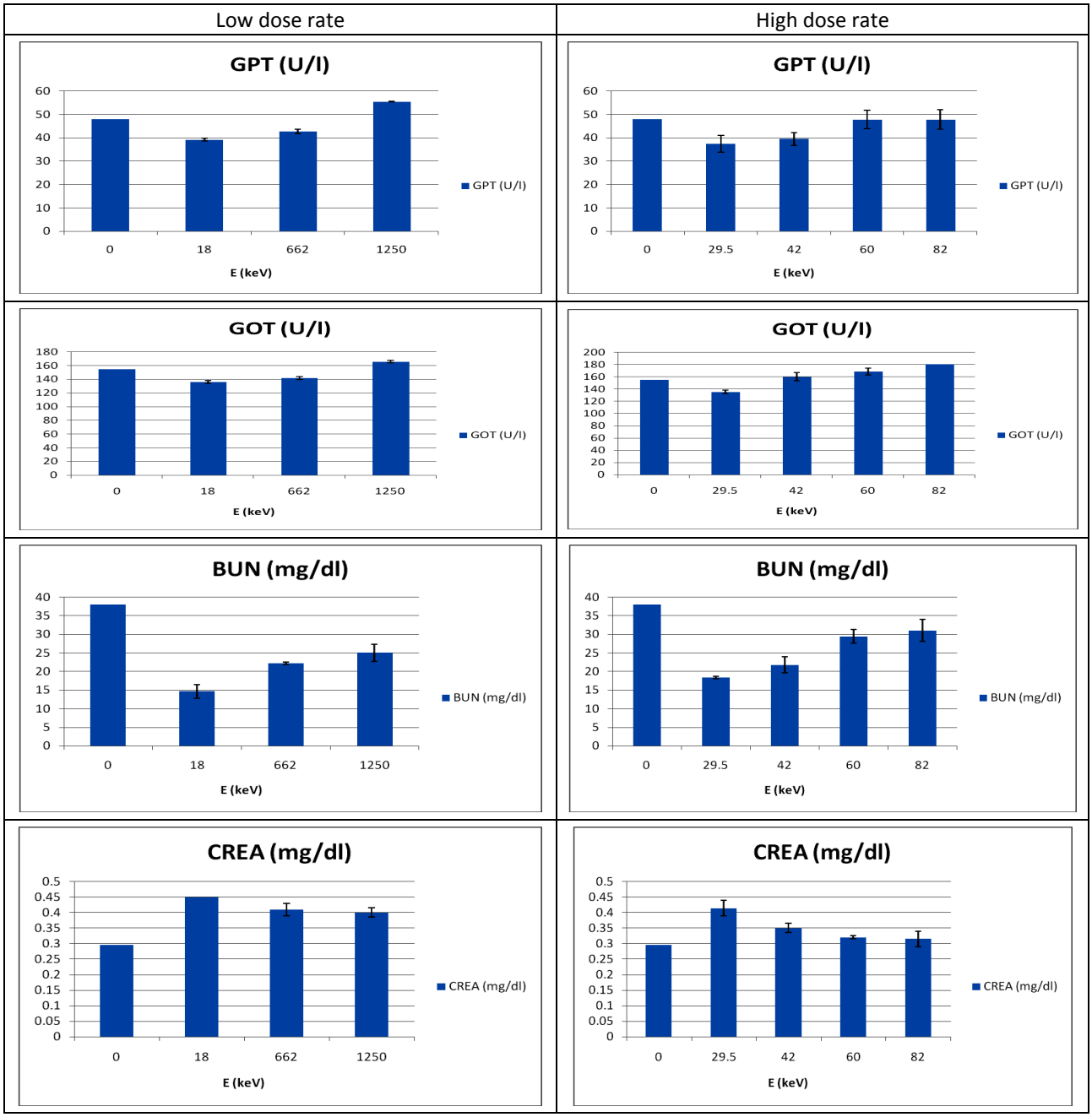
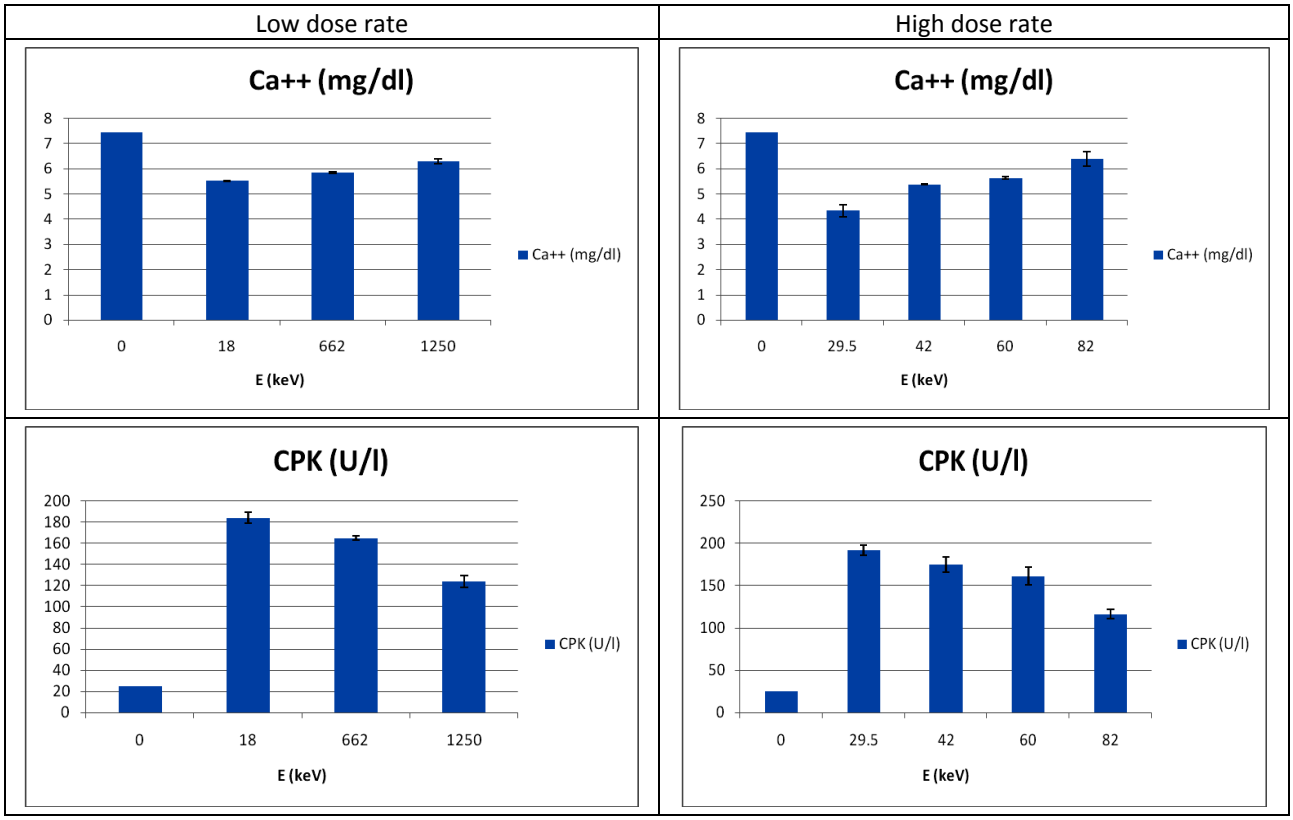


Figure 2. Representation for the SGPT, SGOT, BUN, CREA, Ca++ and CPK levels in blood for rats exposed to same dose with different energies.



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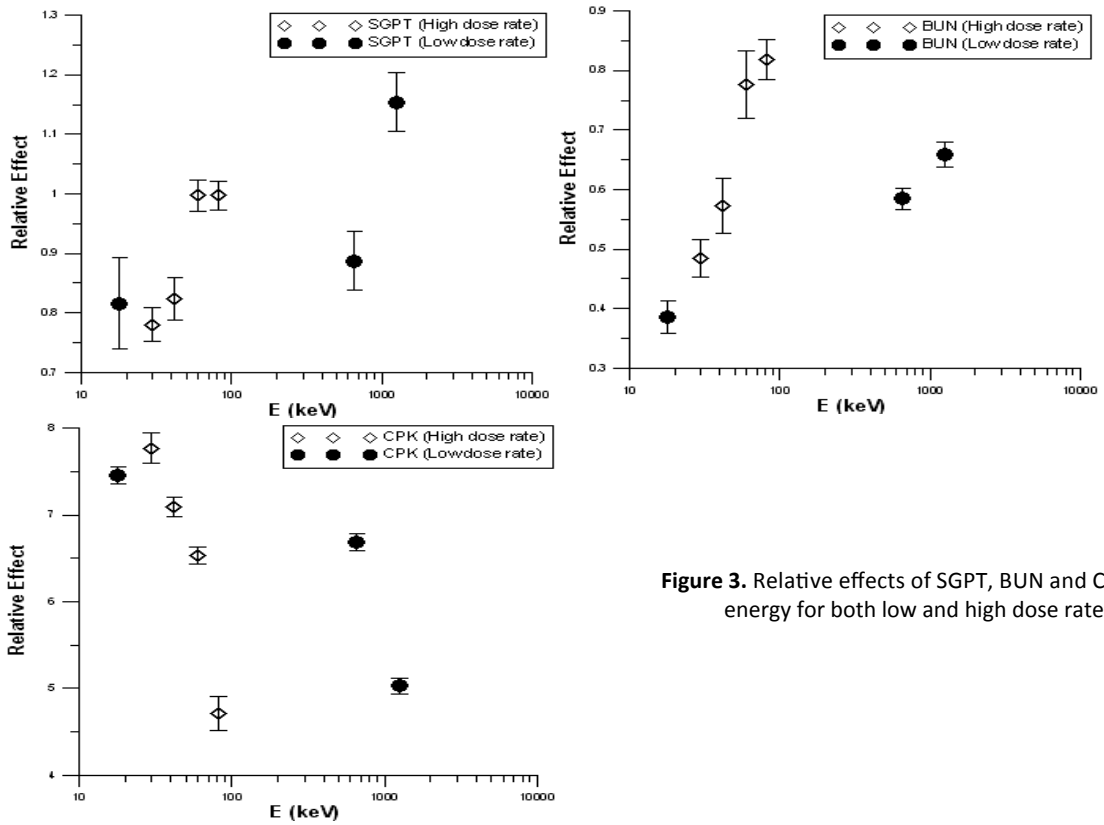

Figure 3. Relative effects of SGPT, BUN and CPK with energy for both low and high dose rates.

DISCUSSION

From table 1, the first HVL for the studied X-ray beam qualities were in agreement with that of CCRI beam quality in which NIS ion chamber was calibrated that reflects accurate X-ray dosimetry. One can notice from table 2 that the studied beams cover two orders of magnitude of X-ray energies up to 100 keV beside the photon energies of ^{137}Cs (662 keV) and ^{60}Co (1250 keV) that gives ability of studying a wide range of energy. From the obtained data, one may conclude that using radiation dose of 50 mGy for studying energy dependence of the biological effect is very convenient due to significant resulting variations of exposed group compared to control animals. This dose was chosen to overcome cell modification and to avoid radio- sensitivity and/ or cell repair capacity in the low dose region [17-19].

According to the present results sera AST and ALT levels showed decreased enzyme levels that were highly significant for exposure to low energy photons as compared with those received higher photon energies. Levels of BUN showed severe decreased values for all exposed groups, whereas very highly significant decrease was shown for exposure to lowest energy photons. This finding is in a perfect accordance with the results of the liver enzyme findings since BUN is a by-product from protein metabolism by liver.

Renal creatinine for the present study showed highly elevated levels for all exposed groups, whereas very highly significant increase was recorded for exposure to lowest energy photons. A rise in blood creatinine level is observed only with marked damage to functioning nephrons [20]. These highly augmented levels of sera CREA reflect convenience pathological injury of the renal nephrons due to direct effect of radiation exposure on levels of cellular, mechanical and physical structure.

Very highly significant increased sera CPK levels were measured after all experimental exposures, whereas very highly significant increase was recorded for exposure to lowest energy photons. Calcium ions (Ca^{+2}) play an essential role in normal cell function whose molecules work to attract other needed molecules within the cells metabolism process [21]. Our data revealed decreased levels of Ca^{+2} for all exposed groups as compared by control. It is hypothesized that exposure to ionizing radiation triggered the disturbance in the cell membrane receptors resulting in reduced endoplasmic reticulum Ca^{+2} concentration.

From figure ,2we notice a significant variation of the measured biochemical parameters with photon energy. It is obvious that the numerical value behavior of the measured parameter tends to control value with energy increase which reflects that the hazard in low energy is much more than high energy ones.

According to the well-known equation that the effective dose is given by (ICRP 119):

$$\text{Effective dose} = \sum_T w_T \sum_R w_R D_{T,R} \quad \text{or} \quad E = \sum_T w_T H_T \qquad (2)$$

Where H_T or w_R $D_{T,R}$ is the equivalent dose in a tissue or organ, T, and w_T is the tissue weighting factor.

Since the w_T and H_T in the above equation are constants and the absorbed dose used in this study has the same value for all irradiated groups then the same effect may be produced whatever the energy of irradiation that is contradicting with the obtained results as seen in figure 3.

One more important point worthy to be mentioned is that exposure to low dose rate is more biologically effective than exposure to high dose rate. This could be attributed to the oxygen molecule interaction with the free radicals and active sites induced by ionizing radiation which will inhibit recombination and cross linking reactions [22].

CONCLUSION

From the present work, one may conclude that by using the added filtration method, precise X-ray beam qualities were obtained as that of the CCRI. By the aid of HVL in Cu,

effective X-ray energy was accurately determined. The accurately obtained X-ray dose and energy gives the ability to study the biological effects energy dependency. It's clear from the data that biological effect is energy dependent and radiation hazards induced from the same absorbed dose is of wide variation. Low energy photon produces more hazard than higher ones. So it is recommended to reevaluate the methods used for the effective dose and ambient dose calculations on bases of *in-vivo* study especially with low energy X-ray.

Conflicts of interest**:** *This work is a part of the Ph.D. thesis of Reham Hamdy Bakr submitted to the Biophysics Department, Faculty of Science, Cairo University.*

The authors declare that they have no conflict of interest.

No external fund was given and/or available to run this work.

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