

# Evaluation of low dose diagnostic X-rays induced effect on the white blood cells count in Guinea pigs

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## ABSTRACT

### ► Short report

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**Background:** Exposure to ionizing radiation is known to have various effects on hematological parameters of biological sample. This study was aimed at evaluating the effect of ionizing radiation on some hematological parameters in guinea pigs.

**Materials and Methods:** Thirty-six (36) apparently healthy adult guinea pigs of both sexes weighing between 700g – 1200g were used in the present study. The guinea pigs were categorized into three groups, 12 per group; group A (control), group B, and C were exposed to X-rays within the diagnostic range, using 70 kV and 12.5mAs; using X-ray machine MS-185, serial no. 0904 GE at a source to skin distance (SSD) of 90cm. Blood samples were collected from all the guinea pigs at intervals of 1 hour, 24 hours 72 hours, 168 hours and 336 hours post-irradiation, and subjected to standard hematological analysis. **Results:** A continuous decline in the mean total white blood cell count and mean lymphocyte, monocyte, neutrophil and eosinophil count after 1hour in both groups was observed, and more pronounced after 24 hours post-irradiation. However, stability was observed 72 hours post-irradiation in both groups. **Conclusion:** This study found a depleting effect of low dose ionizing radiation on white blood cell count, with appreciable recovery occurring after 72 hours onward.

**Keywords:** White blood cells, irradiation, Guinea pigs, ionizing radiation, hematological parameters.

## INTRODUCTION

Ionizing radiation is widely used in the medical field for both diagnostic and therapeutic purposes in form of X-rays, gamma rays, and particles ( $\alpha$ -particle,  $\beta$ -particle, protons and neutrons) radiations <sup>(1)</sup>.

The effect of ionizing radiation on the biological tissue arises from the deposition energy in the tissues which can cause changes in the chemical composition of the cell. The energy of the ionizing radiation is significantly greater than the bond energies of many molecules and can cause hemolytic bond scission and generation of secondary electrons <sup>(2)</sup>.

Ionizing radiation is thus seen to affect biological tissues by directly dissociating molecules following their excitation and ionization, or indirectly by the production of free radicals and hydrogen peroxide in the water of the body fluids<sup>1</sup>, and the severity of the effect increases with dose and dose rate <sup>(3)</sup>.

Although the application of radiation involves a certain level of risk, the use of radiation in medicine results in such numerous benefits that if judiciously employed, the benefits greatly exceed the risk to the individual <sup>(4)</sup>. The hematopoietic system is highly sensitive to radiation, and peripheral blood examination may serve as a biological indicator of such

damage that may occur even at very low doses of ionizing radiations like X-rays or gamma rays<sup>(1,5)</sup>. Peripheral blood examination may serve as a screening test for various hematological as well as non-hematological disease state<sup>(5)</sup>.

The major functions of the white blood cell and its differentials are to fight infections, defend the body against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response<sup>(6)</sup>. The decrease in the white blood cell count leaves the individual at risk of infection. Low white blood cell count is known as leucopenia<sup>(7)</sup>. It has been observed that there is always a slight decrease in the total white cells count after the first few days of exposure to ionizing radiation; hence, white blood cells count may be a reliable indicator of the degree of exposure<sup>4</sup>. Irradiating animal models to a single whole-body dose of ionizing radiation results in complex sets of symptoms whose onset, nature and severity are a function of both total radiation dose and radiation quality which classified into three syndromes; the hematopoietic syndrome, the gastrointestinal syndrome and the central nervous syndrome. The hematopoietic syndrome occurs at very low radiation doses and is manifested by depletion of hematopoietic stem cells and ultimately by depletion of matured haemopoietic and immune cells<sup>(8)</sup>.

This study is looking closely at the changes that may occur on white blood cells count after exposure to ionizing radiation (X-ray) within the diagnostic range of guinea pigs as an animal model.

## **MATERIALS AND METHODS**

Institutional approval to conduct the study was obtained from the committee on ethics of the Veterinary teaching hospital, University of Maiduguri (VTH). Thirty-six guinea pigs were obtained and kept at the large animal clinic of the VTH, Faculty of Veterinary Medicine University of Maiduguri, under good ventilation and adequate light. The guinea pigs were fed with standard commercial prepared diet (pelletized feed) and vegetables (such as

cabbage and carrots) and given free access to clean drinking water. The guinea pigs were kept in this condition for fourteen days in order to acclimatize before starting the experiment<sup>(9)</sup>. The guinea pigs were routinely screened for ectoparasites, endoparasites and haemo-parasites using standard methods by a veterinary doctor, and randomly divided into three groups, 12 guinea pigs per group. Group A served as the control group, group B and group C were exposed to low-dose X-rays at a dose that is within the diagnostic range, using factors for chest X-ray of an adult patient in the study centre (70kV and 12.5mAs) using X-ray machine MS-185, serial no. 0904 GE, on which quality assurance check was routinely performed by a medical physicist with over 8 years experience, at a source to skin distance (SSD) of 90cm. The guinea pigs in each group were irradiated together using a vertical central ray on a horizontal table top (couch) within the same cage, with the radiation properly collimated to include all the guinea pigs. Group C was irradiated twice with the same exposure factors five minutes after the first exposure.

### **Blood sample collection**

Blood sample from each guinea pig was collected into EDTA bottle from direct cardiac puncture with a 2ml syringe and appropriately labelled. The blood samples were collected at the intervals of 1hour, 24hours, 72hours, 163hours and 336 hours post irradiation of the experimental groups. Blood samples were also collected during the same time interval from the control group. No same syringe was used to collect blood sample more than once. The blood cell count was done by a veterinary doctor with over 10 years experience in veterinary parasitology at the veterinary teaching hospital, University of Maiduguri, who performed the procedure alone to avoid inter-observer error. Hemocytometric method was used to count the white blood cells using Neubauer counting chamber. This method was used due to availability and convenience, as the automatic analyzer was not readily available at the time of analysis.

### Statistical analysis

The mean values of hematological parameters of control, single and double exposure groups were determined using one-way analysis variance (ANOVA). P-values <0.05 was considered significant and the mean  $\pm$  SE for hematological parameters were presented using descriptive statistics.

## RESULTS

Table 1 shows the mean  $\pm$  SE of white blood cell count values of guinea pigs following single and double exposure to x-ray within diagnostic range.

There was an observed decline in the mean total white blood cell count of guinea pigs at 1hour after single and double exposure. This decrease was sustained and more pronounced 24 hours post irradiation. However, recovery of WBC commenced at 72 hours post exposure in both single and double exposure groups. This was found to be significant ( $P < 0.05$ ) as shown in table 1.

An observed decline in the mean absolute monocyte count at 1hour post irradiation was also noted. This decrease was more pronounced at 24 -72 hours post irradiation. However, recovery of monocytes was evident at 168 -336

hours post irradiation in both single and double exposure groups, with no significant difference ( $P < 0.05$ ) as shown in table 1.

There was also an observed slight decline in the mean absolute lymphocyte count of guinea pigs at 1hour in the exposure groups, which was more pronounced 24 -72 hours post irradiation in both exposure groups. However, recovery of the mean absolute lymphocyte count was evident at 168 and 336 hours post irradiation as shown in table 1.

There was an observed decline in the mean absolute Eosinophil count of guinea pigs in both single and double exposure group at 1hour following single and double exposure to irradiation. This decrease was sustained and was more pronounced 24 -72 hours post irradiation. However, there was a slight recovery of the mean absolute eosinophil count at 168 -336 hours post irradiation, as shown in table 1.

An observed decline in the mean absolute neutrophil count of guinea pigs at 1hour in both single and double exposure groups, and became more pronounced 24 hours after irradiation. However, slight recovery of the mean absolute neutrophil count in guinea pigs was observed at 168-336 hours post irradiation as shown in table 1.

**Table 1.** Effects of low radiation dose exposures on haematological parameters of Guinea Pigs (*Cavia porcellus*).

PARAMETERS	Mean $\pm$ SE																	
	CONTROL GROUP						SINGLE EXPOSURE						DOUBLE EXPOSURE					
	0	1hr	24hr	72hr	168hr	336hr	0	1hr	24hr	72hr	168hr	336hr	0	1hr	24hr	72hr	168hr	336hr
WBC	10.4 $\pm 0.8$	10.7 <sup>a</sup> $\pm 0.8$	10.2 <sup>a</sup> $\pm 0.6$	10.5 <sup>a</sup> $\pm 0.5$	11.0 $\pm 1.0$	10.7 $\pm 0.4$	10.5 $\pm 0.5$	8.7 <sup>a</sup> $\pm 0.9$	5.8 <sup>b</sup> $\pm 0.8$	6.2 <sup>b</sup> $\pm 0.9$	8.0 $\pm 1.1$	8.3 $\pm 1.2$	11.0 $\pm 0.4$	8.7 <sup>a</sup> $\pm 0.4$	6.1 <sup>b</sup> $\pm 0.2$	6.1 <sup>b</sup> $\pm 0.2$	8.9 $\pm 0.3$	9.3 $\pm 0.3$
PCV	40 $\pm 1.8$	40.3 $\pm 1.8$	40.5 $\pm 1.5$	38.5 $\pm 1.9$	38.8 $\pm 0.8$	39.3 $\pm 1.4$	40 $\pm 1.1$	35.3 $\pm 3.3$	28.9 $\pm 4.0$	27.4 $\pm 3.8$	30.3 $\pm 4.2$	31.6 $\pm 4.4$	41 $\pm 1.1$	38.5 $\pm 1.0$	31.8 $\pm 1.0$	34.3 $\pm 1.3$	38.0 $\pm 1.2$	39.5 $\pm 0.9$
HB	13.0 $\pm 0.4$	12.8 $\pm 0.9$	12.8 $\pm 0.9$	12.5 $\pm 0.3$	12.3 $\pm 0.3$	12.3 $\pm 0.3$	12.6 $\pm 0.3$	11.2 $\pm 1.1$	9.0 $\pm 1.2$	8.8 $\pm 1.2$	9.3 $\pm 1.3$	10.0 $\pm 1.4$	13.1 $\pm 0.3$	11.9 $\pm 0.5$	9.8 $\pm 0.4$	10.8 $\pm 0.3$	11.7 $\pm 0.3$	12.3 $\pm 0.2$
Monocyte	0.4 $\pm 0.2$	0.3 <sup>a</sup> $\pm 0.1$	0.3 <sup>a</sup> $\pm 0.1$	0.4 <sup>a</sup> $\pm 0.1$	0.4 <sup>a</sup> $\pm 0.1$	0.3 <sup>a</sup> $\pm 0.1$	0.3 $\pm 0.0$	0.2 $\pm 0.0$	0.2 <sup>b</sup> $\pm 0.0$	0.1 <sup>b</sup> $\pm 0.0$	0.2 <sup>b</sup> $\pm 0.0$	0.2 <sup>b</sup> $\pm 0.0$	0.3 $\pm 0.1$	0.1 <sup>b</sup> $\pm 0.0$	0.1 <sup>b</sup> $\pm 0.0$	0.1 <sup>b</sup> $\pm 0.0$	0.2 <sup>b</sup> $\pm 0.0$	0.2 $\pm 0.0$
Lymphocyte	4.7 $\pm 0.7$	5.2 $\pm 0.6$	4.9 <sup>a</sup> $\pm 0.5$	4.7 <sup>a</sup> $\pm 0.6$	4.9 $\pm 0.7$	4.7 $\pm 0.4$	3.7 $\pm 0.5$	4.0 $\pm 0.5$	2.5 <sup>b</sup> $\pm 1.3$	2.6 <sup>b</sup> $\pm 0.4$	3.2 $\pm 0.5$	3.7 $\pm 0.6$	6.0 $\pm 0.4$	4.4 $\pm 0.3$	2.9 <sup>b</sup> $\pm 0.2$	2.7 <sup>a</sup> $\pm 0.1$	3.8 $\pm 0.2$	4.7 $\pm 0.3$
Neutrophil	5.5 $\pm 0.7$	5.7 $\pm 0.8$	5.2 <sup>a</sup> $\pm 0.6$	5.2 <sup>a</sup> $\pm 0.5$	5.7 $\pm 0.8$	5.5 $\pm 0.3$	5.5 $\pm 0.4$	4.3 $\pm 0.6$	2.7 <sup>b</sup> $\pm 0.0$	2.8 <sup>b</sup> $\pm 0.5$	3.8 $\pm 0.6$	4.2 $\pm 0.6$	5.8 $\pm 0.3$	4.3 $\pm 0.3$	2.8 <sup>b</sup> $\pm 0.1$	2.7 <sup>b</sup> $\pm 0.1$	4.1 $\pm 0.2$	4.5 $\pm 0.2$
Eosinophil	0.4 $\pm 0.07$	0.4 <sup>a</sup> $\pm 0.1$	0.4 <sup>a</sup> $\pm 0.1$	0.4 <sup>a</sup> $\pm 0.1$	0.4 <sup>a</sup> $\pm 0.1$	0.4 <sup>a</sup> $\pm 0.06$	0.4 $\pm 0.03$	0.2 <sup>b</sup> $\pm 0.0$	0.1 <sup>b</sup> $\pm 0.4$	0.1 <sup>b</sup> $\pm 0.0$	0.1 <sup>b</sup> $\pm 0.0$	0.1 <sup>b</sup> $\pm 0.0$	0.5 $\pm 0.04$	0.2 <sup>b</sup> $\pm 0.0$	0.1 <sup>b</sup> $\pm 0.0$	1.1 <sup>b</sup> $\pm 0.0$	0.2 <sup>b</sup> $\pm 0.0$	0.2 <sup>b</sup> $\pm 0.0$
Basophil	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00

## DISCUSSION

The study found a decrease in total white blood cell count; lymphocytes, monocytes, neutrophils, eosinophil and absence of basophils. Basophils naturally are rarely encountered granulocytes in the peripheral blood; therefore it is not unusual for basophils to be absent<sup>10</sup>. Similar findings were previously reported by (2, 4, 11). The observed decrease in the white blood cell counts could be attributed to high radio-sensitivity of hematopoietic tissue (2, 12). The results are consistent with the previous findings that irradiation induces leukopenia and reduces lymphocytes, neutrophils and monocytes count (13, 14). However, the recovery was evident 72 hours post irradiation and onward, even though the recovery and repair took longer time than the damage<sup>4&14</sup>. This could be due to the fact that the recovery might be as a result of the repair at the cellular level where sub-lethally damaged cells recover their viability and by the proliferation of undamaged cell elements (4, 11). The effect on the double exposure group was severe, which proves the fact that severity of damage increases with increase in dose or exposure (3, 11, 14).

## CONCLUSION

This study found a depleting effect of low dose ionizing radiation within diagnostic range on white blood cell counts of Guinea Pigs (*Cavia porcellus*). This was found to be more pronounced with repeated exposures. However, recovery occurred from 72 hours post irradiation onwards, which was found to be slower than the rate of damage. Thus, a proposed interval of 72 – 336 hours before repeating an exposure is recommended for subjects that may require a series of follow-up and repeat radiographic examinations.

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