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Standardization of Mean Lethal Dose (LD $_{50/30}$) of X -rays using Linear Accelerator (LINIAC) in Albino Wistar Rat Model Based on Survival Analysis Studies and Hematological Parameters.

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ABSTRACT

This study aims at determining the dose of X-ray delivered through linear accelerator (LINIAC) that results in the 50 % survival of animals after an observation period of 30 days, following exposure to whole body irradiation in rat model. Animals were exposed to several doses of radiation (2, 4, 6, 8, 10 Gy) after which they were monitored for survival rate, overall behavior, diarrhoea and skin lesions. Animals of either sex were placed in a Perspex box restrainer and exposed to whole body irradiation of X -rays. Whole body Irradiation of X- rays was carried out by LINIAC accelerator. Radiation ranging from 2 to 10 Gy was delivered to rats with Dose rate of 3.5 Gy/min with field size of 40 × 40 cm^2. Hematological parameters like total count, differential count and peripheral smear examination was carried out from the peripheral blood of the animals to complement with survival studies and compared with controls. Peripheral smear examination was done by Leishman's staining method. Based on the results of our study, we report that the LD $_{50/30}$ of Wistar rats exposed to whole body irradiation of X–rays through linear accelerator was found to be 6.6 Gy. Whole body exposure of Ionizing radiation leads to complex cascades of syndromes which depends on the nature of radiation dose and quality. Establishing LD $_{50/30}$ in experimental animals will support further studies on the beneficial effects of the radio modulators that could probably reduce the morbidity and mortality in animals exposed to radiation.

Keywords: Mean Lethal Dose, Radiation, Probit analysis, Ionizing radiation, X Rays.

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INTRODUCTION

Ionizing radiation can result in mutation, radiation sickness, cancer and eventually death of an organism due to the damage caused to the living tissue. These ill effects may be minimized by treating the organism using chemical and herbal modulators [1]. A systemic and detailed study on the role of these compounds in modulating the effect of radiation, essentially requires the determination of $LD_{50/30}$ of radiation i.e., the mean lethal dose that causes 50 % death in animals exposed to radiation [2] ,so that further studies may be performed using sub lethal dose. The effect of X-rays on lymphatic tissues and a consequential reduction in circulating lymphocytes was first observed by Heineki and later confirmed by Warthin's work on lymph glands and bone marrow in animal studies [3,4] following which, there were a number of studies reporting radiation induced immunosuppression which is predominantly reflected as leukopenia [5,6]. The present study therefore aims at studying the mortality rate, using hematological parameters to complement the survival/death in post irradiated animals following exposure to serial dose of x rays.

MATERIALS AND METHODS

Animal care and handling was carried out according to the guidelines set by WHO. Adult (8-10 weeks old) male or female wistar rats were used in the present study. The animals were procured from the breeding colony of Kasturba Medical College Animal House, Mangalore. Prior approval of Institutional animal ethical committee was taken. Animals were housed in polypropylene cages with a paddy husk bedding at 25 ± 2^0C temperature during the experiment. Rats were given an ad libitum access to laboratory food (commercial rat pellets from Gold Mohr India Ltd) and water. 72 wistar rats, aged 8 -10 weeks, weighing 150 ±10 gram were divided into six groups with twelve animals in each group. The animals were placed in a well-ventilated Perspex box rectangular restrainer of size 24 × 18 centimeters and wall thickness of 1 mm. Whole body irradiation was administered to the experimental animals by LINIAC accelerator having a field size of 40 cm× 40 cm^2 with a dose rate of 3.5 Gy/minute and a distance from source to subject of 100 centimeters. The instrument delivers high energy X- rays which penetrate deep into the tissues. The Dosimetry of LINIAC accelerator was carried out by International atomic energy agency (IAEA), Dosimetry and Medical Radiation Physics Section, Austria. The radiation exposure was carried out at Department of Radiotherapy, KMC Hospital, Attavar, Mangalore. Each group of experimental animals were administered with either 2 Gy, 4Gy, 6Gy, 8Gy or 10 Gy of radiation. The group that was not irradiated served as control.

Survival assay

Animals were observed for 30 days post irradiation. They were monitored for anorexia, weight loss, facial edema, hair texture, overall behavior, skin lesions and diarrhoea. Number of animals that survived in each group post radiation for a period of 30 days was recorded. The mean lethal dose was calculated by probit analysis.

Total and differential Leukocyte count

Blood was withdrawn from experimental animals by cardiac puncture 3rd day post irradiation and peripheral smear was performed using standard staining procedures [7].

Statistical analysis

$LD_{50/30}$ was calculated by probit analysis. Analysis of Blood cell count between the groups was done by one way analysis of variance (ANOVA) followed by Post Hoc test, using IBM SPSS Statistics 20. $p \leq 0.05$ was considered significant. Kaplan Meir method was used to study survival.

RESULTS

Percentage mortality in various groups were as follows,0 % mortality was observed in 2 Gy and 4 Gy, 16.6% mortality at 6 Gy and 100 % mortality at 8 and 10 Gy. Animals exposed to 8 Gy died in 11 days post irradiation while those exposed to 10 Gy died within 7^{th} days post irradiation with severe radiation sickness such as anorexia, weight loss, diorrhoea, facial edema and ruffled hair. Animals were unaffected at 2 and 4 Gy but slight anorexia was seen at 6 Gy. The percentage mortality of each dose of radiation was transformed to

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probits [Table 1].The probit values were plotted against the log dose of radiation. The dose corresponding to probit 5 is found out. In this experiment it is Log 0.82 [Figure 1] .LD $_{50/30}$ of X rays in wistar rats was found to be 6.6Gy.Kaplan Meier's estimate of rats exposed to different dose of radiation shown in [Figure 2].

Peripheral smear

It was observed that there was a significant decrease in total leukocyte count ($P<0.05$) as well as Lymphocytes, Monocytes, Eosinophils and Neutrophils with increase in the radiation dose [Figure 3] .RBC and WBC did not vary much with respect to morphology.

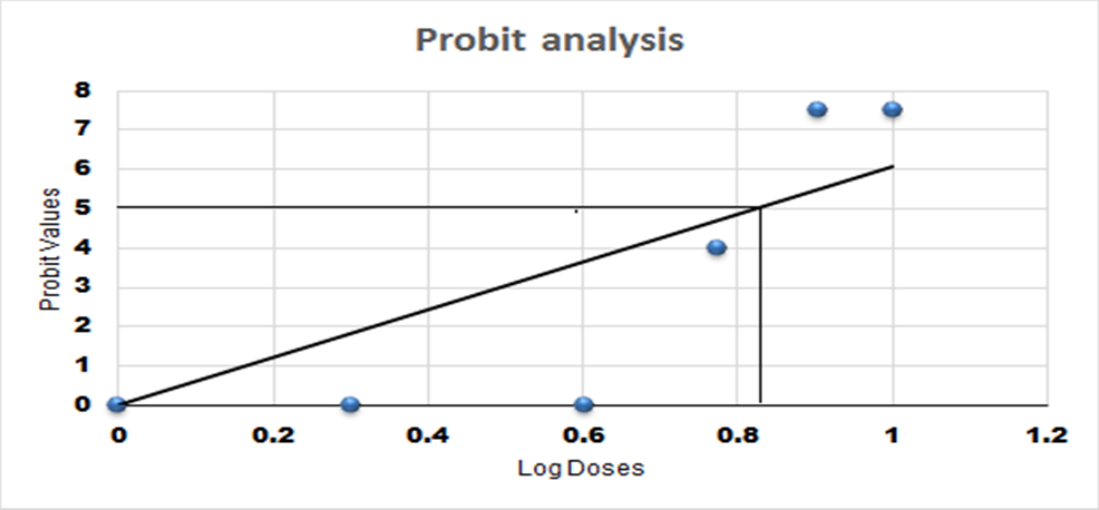

Figure 1: Plot of log dose of radiation versus probit values from table 1 and calculation of LD $_{50}$.

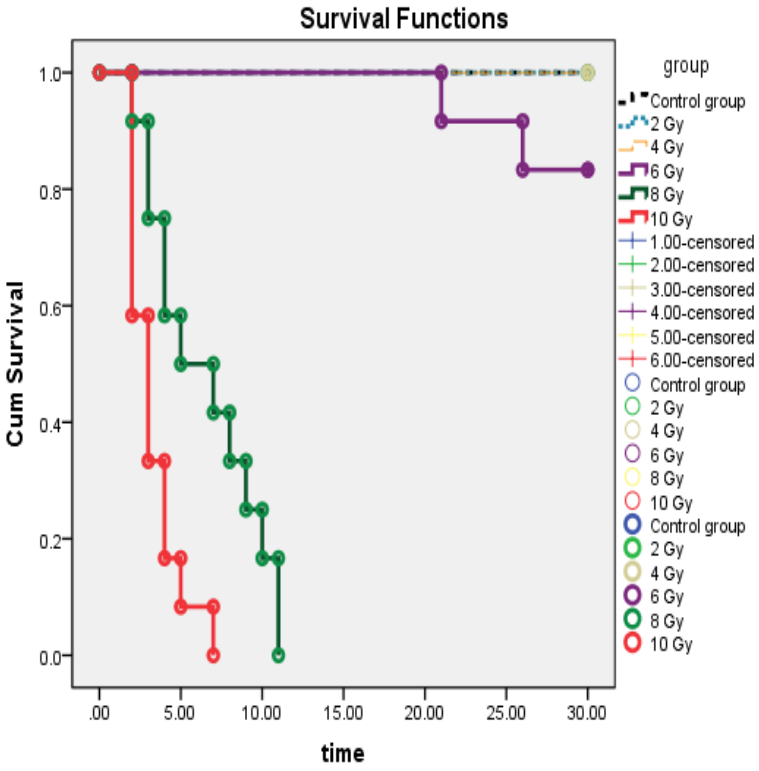

Figure 2: Kaplan Meier's estimate of survival of rats treated with whole body irradiation of different doses of X rays (2-10 Gy).Twelve animals maintained in each group.

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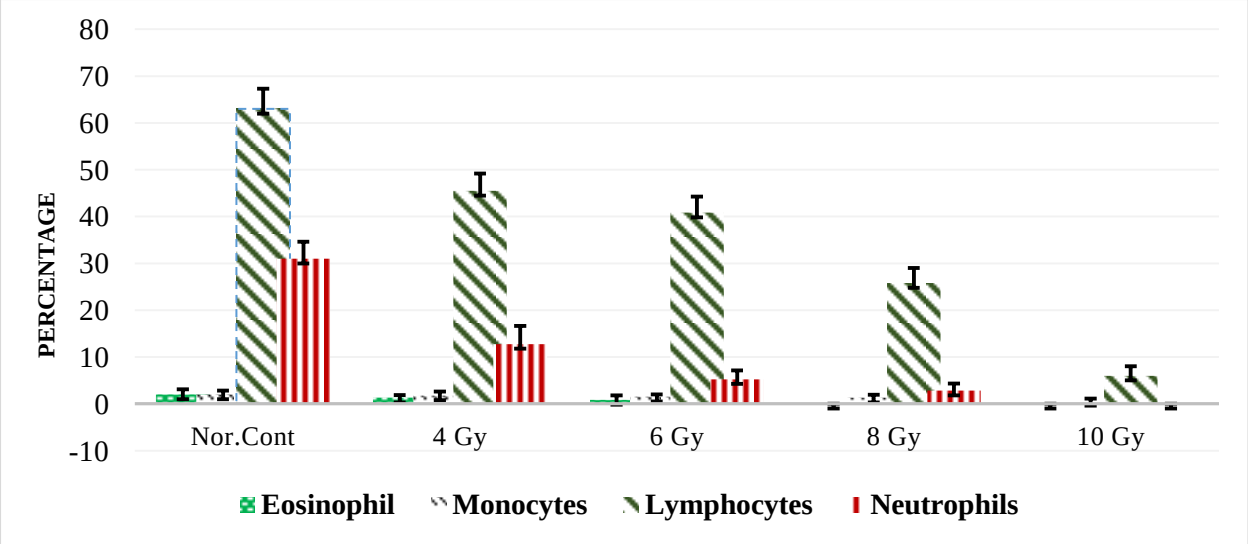

Figure 3: Effect of different dose of x- rays on differential count.

Table 1: Estimation of LD $_{50/30}$ of x rays & conversion of percentage mortalities into probits.

GY	LOG DOSE	PERCENTAGE MORTALITY (%)	PROBIT VALUE
2	0.301	0	0
4	0.602	0	0
6	0.744	16.6%	4.01
6	0.903	100 %	7.5
10	1	100 %	7.5

DISCUSSION

Ionizing radiation can damage the tissues either by directly attacking the nucleus causing double strand breaks (direct effect) or attack the water content in the cytoplasm of cell generating free radicals which in turn cause DNA damage [8] (Indirect effect).LD $_{50/30}$ for Swiss albino mice was reported to be 6.5-9.5 Gy by Uma Devi P et al [2]. Certain other studies have reported it to be 10 Gy [9]. Information regarding LD $_{50/30}$ in wistar rats is limited. In this context, we report the LD $_{50/30}$ for wistar rats as 6.6 Gy using high energy X- rays delivered through linear accelerator as determined by Probit Analysis [10]. X rays are used to treat deep seated tumors since they have high penetrating power. A dose of 2 and 4 Gy had no effect on the experimental animals as the studied parameters were unaffected although there was a significant decrease in the differential count as compared to normal controls ($P<0.05$).However a dose of 6 Gy caused slight anorexia. Follow up observation of post treated animals with 6 Gy, 8 Gy and 10 Gy correlated with the findings of peripheral smear which in turn was dose dependent. This indicates that bone marrow suppression is a hallmark of radiation treatment, as rapidly dividing cells are most radiosensitive. As stated by earlier studies, this could be attributed either to depletion of B cell lineage subsets and generalized apoptosis in bone marrow cells [11] or alteration in gene and protein expression in bone marrow followed by protein oxidation reducing cell viability during radiation treatment [12].

CONCLUSION

We establish LD $_{50/30}$ (Mean Lethal Dose) of Wistar rats exposed to X- rays as 6.6. Gy based on probit analysis which was supported by the observations of peripheral smear.

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