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Neutrophils Response in Mice to Low Level Gamma Irradiation and Their Protection by Liv.52

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ABSTRACT

Series of studies were conducted to explore the effects of gamma irradiation to peripheral blood of male Swiss albino mice. Radioprotection was evaluated by the ability of Liv 52 to reduce the lethality produced by cobalt-60 gamma radiation. Mice were treated by oral gavage once daily for seven consecutive days with Liv 52 (500 mg/kg body weight) prior to radiation.Male Swiss albino mice were exposed to 1 and 3 Gy of whole-body gamma irradiation in the presence (experimental) and absence (control) of a herbomineral formulation of Liv.52. Quantitative variations in the number of total leukocytes count (TLC), lymphocytes and neutrophils were scored in peripheral blood at various time intervals between on the day of exposure to 28 days. At 1Gy dose, depression in TLC was noticed till day 1, whereas in higher doses until day 5 with a sharpness in first 24 hrs. Prior administration of Liv.52 significantly prevented the depletion of leukocytes count and initiated recovery towards normal at 28 days in experimental animal. The behavior of neutrophils was reciprocal as they showed rise till day 1 followed by gradual decline up to day 5 in control (without liv 52) as well as in experimental group with both the irradiation doses. It is noted that liv 52 decreases the direct cell killing against gamma radiation may be due to by increasing the cellular glutathione (GSH) level [9] and restores early recovery of lymphocyte in drug treated animal.

Keywords: Differential leukocytes count, Mice, Liv.52, Gamma Rays, Radioprotection.

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INTRODUCTION

The twentieth century has seen an increasing use of nuclear energy in industrial, medical, engineering and scientific research that have raised the problem of radiation hazards to living beings. Thus, the development of effective radio protectors and radio recovery drugs is of great importance in view of their potential application during both planned (i.e., radiotherapy) and unplanned radiation exposure (i.e., in the nuclear industry, natural background radiation) [1].Radiation-induced hematological alterations have been extensively studied. Lymphocytes are among the most radiosensitive cells in the living organisms [2]. They are involved in immunological responses and are of immense interest to researchers and clinicians, because of their extreme sensitivity to ionizing radiation [3,4].Extensive research has been carried out in recent years to find a suitable chemical radio protective agent, which can be administered safely before radiation exposure. Several chemical compounds like cystein, cysteamin, 2-mercaptopropionyl glycinehave been known to afford a high degree of protection against radiation in mammals, but most of them were found toxic at their optimum protective dose level [5] Liv.52 was revealed to be a non-toxic, hepatoprotective as well as radio protective drug [3,9,12] .This study has done to investigate the protective efficacy of this drug against radiation-induced quantitative variations in differential leucocytes count of peripheral blood in mice [6-8].

MATERIALS AND METHODS

Animals

Young adult male Swiss albino mice of 6-8 weeks age weighing about 20 ± 2 gms were selected from a closely bred colony maintained on standard mice feed (procured from Hindustan Lever Ltd., India) and water *ad libitum*.. The selected mice were divided in two different groups. One group of animals was orally given a 5% dextrose solution once a day for 7 days before irradiation to serve as control while the other group received 500 mg/kg body weight of Liv.52 powder (The Himalaya Herbal Drug Co. Mumbai) dissolved in 5% dextrose solution in a similar manner to serve as experimental group.

Irradiations

One hour after administration on day 7, the animals of both control and experimental groups were exposed to two different sub lethal doses (1 Gy and 3 Gy) of gamma radiation. The animals were whole-body exposed to gamma radiation by Cobalt teletherapy unit (Co-60) source (dose rate= 1.16 Gy/min) at a distance of 80 cm, at the Radiotherapy Department, Sushrutha Cancer Hospital, Karimnagar. A.P.All these groups were observed daily up to 28days for any sign of sickness, behavioral toxicity and mortality. The animals were autopsied on days 12hrs, 1, 3, 7, 14 and 28 post-irradiation intervals for the study of hematological parameters.

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Hematological study

Blood sample was collected from the orbital sinus of mice from respective groups, in a vial containing 0.5 M EDTA. The number of White Blood Cell (WBC), lymphocyte, and neutrophils percentage were determined by adopting standard procedures

Statistical analysis

The Student's't' test was used for statistical comparison between the groups and significance level was set at different levels as $p<0.05$.

RESULTS

The results obtained from the present investigation are depicted in the Table. The leucocytes in general showed an initial decline after irradiation in both the dose level used. The depletion in count was more rapid during first 24 hours; thenceforth it increased slowly till day 28 in both control and experimental groups at 1 Gy dose. The normal leucocytes count could not be restored in both the groups even up to the last autopsy interval. However, depression was less marked in drug treated animals and a significant protection was observed at later intervals (Table).

Table-1

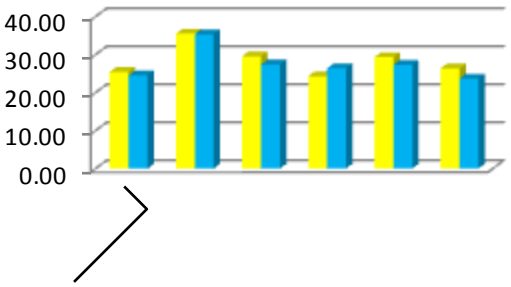
Irradiation Dose (in Gy)	Type of leucocytes	Mode of Treatment	Post-Irradiation Time (In days)					
			12 hrs	1-day	3-day	7-day	14-day	28-day
1 GY	NEUTROPHILS	CONTROL	25.47± 0.13	35.53± 0.16	29.58± 0.24	24.30± 0.15	29.34± 0.11	26.39± 0.15
		EXPERIMENTAL	24.53± 0.15	35.38± 0.21	27.41± 0.28	26.44± 0.17	27.33± 0.14	23.70± 0.17
		p-Value				P<0.05	P<0.05	
3 GY	NEUTROPHILS	CONTROL	30.55± 0.19	39.58± 0.31	30.31± 0.17	25.35± 0.13	31.42± 0.06	29.67± 0.06
		EXPERIMENTAL	28.25± 0.19	38.31± 0.18	31.32± 0.12	24.47± 0.22	29.55± 0.19	27.65± 0.25
		p-Value						P<0.05

At 3 Gy dose, the depletion in number of leucocytes was observed till day 3 and thereafter boosted but remained below normal in both control and experimental groups. The count was significantly higher at later intervals in Liv.52-treated animals.

The variations in lymphocytes number showed a behaviour parallel to total leucocyte count. In 1 Gy group, the percentage of lymphocyte declined in both the groups till day 1 after

which it increased slightly until day 28 and attained normal value in Liv.52-treated animals only at the last autopsy interval. A significant protection in lymphocytes was noticed at day 3and 7th day. (Table).

At 3 Gy, the lymphocyte count depleted till day 1 but the drop was as high as 50 percent of normal (Table). The percentage of lymphocytes showed an increase but the normal count could not be restored till day 28 in both control and experimental animals. A significant protection in lymphocytes was registered on days 7 and 14 with Liv.52.



The neutrophils exhibited a reciprocal bearing as compared to lymphocytes. The latter showed a sharp decline in first 24 hours followed by a slight increased, but the former demonstrated a steep rise during the first 24 hours post-irradiation and then a gradual decline in both control and experimental groups of animals. In 1Gy dose, the percentage of neutrophils increased till day 1, thenceforth decreased up to day 7. In animals treated with Liv.52 prior to irradiation, the number was restored to normal by the last autopsy interval and a significant difference was observed at day 7 and 14. In 3 Gy group, the pattern of neutrophilic variation was similar to the lower dose but not in the Liv.52-treated animals. However, a significant difference in neutrophilic count between the control and the treated groups was noticed on day 28.

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DISCUSSION

In agreement with the findings of earlier worker [2, 5, 6].In the present investigation, a drastic reduction in leucocyte count after irradiation. The leucocytes number showed a drastic decline during the first 24 hours. This initial phase of rapid decrease is due to direct killing of lymphocytes while the slower fall at later intervals in 3 Gy is due to the reduced number of new lymphocytes entering the peripheral blood. The peripheral lymphocytes exhibited a maximum depletion at day 1 in the current investigation elucidating an early cell killing effect of radiations on this cell type, which is the most radiosensitive in peripheral blood. Edmondso [4]and Kumar [10].The change in neutrophilic count was inverse to that of lymphocytes. It increased during first 24 hours, which can be attributed to "abortive" rise in the neutrophils after irradiation. A second peak of neutrophilic elevation was noted on day 14 after irradiation [7,13] suggested that the first peak can be possibly due to hastening of maturation in bone marrow and for the second peak a mobilization phenomenon in response to radiation-induced tissue injury can be held responsible.

In Liv.52-treated animal groups, the total leucocyte count and lymphocyte percentage were higher than the control group. It is evident that Liv.52 diminishes the direct cell killing against gamma radiation by increasing the cellular glutathione (GSH) leveland restores an early recovery of lymphocytes in drug treated animals. It may also be postulated that Liv.52 may increase the amount of excision repair in cells exposed to gamma rays. Biological factors such as repair capacity or structural alterations in the nucleus may be affected by such substance and could be complimentary or additive to the action of free radicals scavenging for protection from radiation-induced damage. A similar protection in lymphocyte count has been observed while using cysteine [14] and MPG [11, 9] in mice prior to irradiation.

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