LEUCOCYTES RESPONSE IN MICE TO LOW LEVEL GAMMA IRRADIATION AND THEIR PROTECTION BY LIV.52

Girish Waghmare¹, Somnath Waghmare*², Ramrao Chavan¹, Dhanraj Mane¹

¹.Department of Zoology, DR.B.A.M. University, Aurangabad, India
².Department of Zoology, Nowrosjee Wadia College of Arts and Science Pune, India

*Email- drsomnathwaghmare@gmail.com

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), 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. 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 [16] and restores early recovery of lymphocyte in drug treated animal.

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

1. 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). Radiation-induced hematological alterations have been extensively studied. Lymphocytes are among the most radiosensitive cells in the living organisms. They are involved in immunological responses and are of immense interest to researchers and clinicians, because of their extreme sensitivity to ionizing radiation⁵. 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 cystein¹⁴, cysteamin¹, 2-mercaptopropionyl glycine¹⁸ have 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. Liv.52 was revealed to be a non-toxic, hepatoprotective as well as radio protective drug. 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.

2. MATERIALS AND METHODS

2.1. 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.
2.2. 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 28 days for any sign of sickness, behavioral toxicity and mortality. The animals were autopsied on days 12 hrs, 1, 3, 7, 14 and 28 post-irradiation intervals for the study of hematological parameters.

2.3. 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) percentage were determined by adopting standard procedures.

2.4. 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.

3. 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). At 3Gy 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 behavior parallel to total leucocytes 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 3 and 7th day. (Table-1). 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.

4. DISCUSSION

In the present investigation, a drastic reduction in leucocytes counts 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. The total leucocytes counts were higher than the control group. A similar protection in lymphocyte count has been observed while using cysteine14 and MPG11, 16 in mice prior to irradiation. Liv.52 provides radio resistance to the bone marrow cells, which possibly accounts for increased number of lymphocytes and hence also for total leucocytes count in drug-treated animals.
Table 1:
Peripheral blood cell changes in mice after exposure to different doses of gamma rays in the presence and absence of Liv.52.

<table>
<thead>
<tr>
<th>Irradiation Dose (in Gy)</th>
<th>Type of leucocytes</th>
<th>Mode of Treatment</th>
<th>Post-Irradiation Time (In days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 hrs</td>
</tr>
<tr>
<td>1 GY</td>
<td>TLC</td>
<td>CONTROL</td>
<td>3233.70 ± 21.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EXPERIMENTAL</td>
<td>3351.00 ± 21.34</td>
</tr>
<tr>
<td></td>
<td>p-Value</td>
<td></td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>3 GY</td>
<td>TLC</td>
<td>CONTROL</td>
<td>2846.30 ± 29.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EXPERIMENTAL</td>
<td>2942.80 ± 31.28</td>
</tr>
<tr>
<td></td>
<td>p-Value</td>
<td></td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Sham Normal values: lymphocyte: 65.05±1 %, TLC: 5815±35/cumm, Neutrophils: 24.05±1.05%. C = only irradiated (Control); E = Liv.52 + irradiated (Treated).

Fig-1

![TLC 1GY Graph](image1)

Fig-2

![TLC 3GY Graph](image2)
It is evident that Liv.52 diminishes the direct cell killing against gamma radiation by increasing the cellular glutathione (GSH) level\textsuperscript{17} and 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.

5. REFERENCES

[26] Moron, M.S., Depiere, J.W. and Mannervik, B. (1979) Levels of GSH, GR and GST activities in

ISSN:0976-0172
Journal of Bioscience And Technology
www.jbstonline.com
rat lung and liver. *Biochemica Biophysica.* 582: 67-78.


