

## Evaluation of Radioprotective Effects of *Rajgira* (*Amaranthus paniculatus*) Extract in Swiss Albino Mice

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### *Radioprotection/Amaranthus paniculatus/CFU-S/LD<sub>50/30</sub>/GSH/LPO/Gamma Radiation.*

The radioprotective efficacy of aqueous extract of *Rajgira* (*Amaranthus paniculatus*) leaves against whole body gamma radiation was studied in Swiss albino mice. The oral administration of *Rajgira* extract at 800 mg / kg body weight / day for 15 consecutive days before whole body exposure to radiation was found to be effective with the LD<sub>50/30</sub> values of 6.33 and 8.62 Gy for irradiation alone and *Rajgira* + irradiation group, respectively, giving a dose reduction factor of 1.36. This effect of *Rajgira* accompanied the increased endogenous spleen colonies and the spleen weight without any side effect or toxicity, as well as the modulation of the radiation-induced decrease of reduced glutathione and the radiation-induced increase in lipid peroxidation assessed in the liver and the blood.

### INTRODUCTION

A search for the chemical agents that are able to protect human beings from ionizing radiation is a key issue in radiation biology.<sup>1)</sup> Radiation produces various pathological changes in living systems and these changes were reduced with the help of certain synthetic chemicals such as cysteine,<sup>2)</sup> cysteamine,<sup>3)</sup> 2-MPG,<sup>4)</sup> WR-2721,<sup>5)</sup> lipoic acid<sup>6)</sup> and deoxyspergualin.<sup>7)</sup> But clinical applications of these compounds are very few owing to their high toxicity at optimum dose level. Recently, the interest has been developed in search for potential drugs, especially, of herbal origins, which are capable of modifying immune and radiation responses without their side effects. Several studies concerning radioprotection have been conducted on vitamins,<sup>8–10)</sup> ginseng,<sup>11)</sup> spirulina,<sup>12)</sup> garlic,<sup>13)</sup> ocimum,<sup>14)</sup> mentha,<sup>15)</sup> ginger,<sup>16)</sup> squalene,<sup>17)</sup> caffeine<sup>18)</sup> and miso (fermented soybean paste).<sup>19)</sup>

Amaranth (*Rajgira*, family-Amaranthaceae) is the world's most nutritious grain. In India, amaranth grain is also called the royal crop or Rama's grain (*Rajgira* and Ramdana). The seeds of *Rajgira* are nutritious and its leaves are an important source of proteins and vitamins, especially, provitamin A ( $\beta$ -carotene) and minerals like calcium and iron.<sup>20)</sup> It was also reported that amaranthus seeds are an alternative natural source of squalene.<sup>21)</sup> Based on the properties and significance of *Rajgira*, the present study has been undertaken to

investigate the radioprotective efficacy of aqueous extract of *Rajgira* leaves against radiation induced sickness, change in body weight, spleen colonies and animal survivability.

### MATERIALS AND METHODS

#### Animals

Male Swiss albino mice (*Mus musculus*), 6–8 weeks old with 25  $\pm$  3 gm body weight from an inbred colony (obtained from Hamadard University, Delhi, India) were used for the present study. Animals were maintained under controlled conditions of temperature (21  $\pm$  10°C) and light (12 hr dark: 12 hr light) in an animal house, and were provided standard mice feed (Procured from Hindustan Lever's Ltd. Delhi, India) and water *ad libitum*.

#### Irradiation

Cobalt teletherapy unit (ATC-C9; Canada) at the Cancer treatment center, Radiotherapy Department, SMS Medical College & Hospital, Jaipur was used for irradiation. Unanesthetised animals were restrained in well-ventilated perspex boxes and exposed whole-body to gamma radiation at the distance (SSD) of 77.5 cm. from the source to deliver the dose-rate of 1.33 Gy/min.

#### *Rajgira* extract (RE)

Plant material *Rajgira* (*Amaranthus paniculatus*, Family Amaranthaceae) collected from Desa (Gujarat) was identified and the specimen placed at Herbarium, Department of Botany, University of Rajasthan, Jaipur. The voucher number is RUBL-19869. Fresh leaves (100 gm) of *Rajgira* were washed, air-dried, powdered and extracted with 1,500 ml of double distilled water (DDW) by refluxing for 36 hrs. (3  $\times$

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12 hrs.) at 40°C. The extract thus obtained was vacuum evaporated so as to make it in powder form and redissolved in DDW just before the oral administration. For the different concentrations, a known amount of extract was suspended in DDW, and 0.1 ml of RE suspension was given to each mouse by oral gavage.

### Experimental design

**Acute drug toxicity:** To determine the acute toxicity of *Rajgira* leaf extract, the animals were divided into 4 groups of 10 each and RE was given orally to them at the concentration of 200, 400, 800 and 1200 mg/kg body weight/day for 15 consecutive days. The mice were observed continuously for 30 days to determine the toxicity of RE in the form of mortality or any other sign, if occurs.

**Determination of optimum dose of *Rajgira* extract against radiation:** For the selection of optimum dose of RE against radiation, animals were given 200, 400, 800 and 1200 mg/kg body weight/day RE for 15 consecutive days. Thirty minutes after the last administration, these animals were exposed to 8 Gy gamma radiation. The reduced glutathione (GSH) and lipid peroxidation (LPO) levels in liver and blood were estimated after 30 minutes of radiation exposure. The optimum dose thus obtained was used for further investigation.

**Reduced glutathione (GSH) assay:** The hepatic level of reduced glutathione (GSH) was determined by the method as described by Moron *et al.*<sup>22)</sup> GSH content in blood was measured spectrophotometrically using Ellmans reagent (DTNB) as a coloring reagent as per the method described by Beutler *et al.*<sup>23)</sup> The absorbance was read at 412 nm using a UV-VIS Systronics Spectrophotometer [Model No. 108, Naroba, India].

**Lipid peroxidation (LPO) assay:** The lipid peroxidation level in liver and serum was measured in terms of Thiobarbituric Acid Reactive Substances [TBARS] by the method of Ohkhawa *et al.*<sup>24)</sup> The absorbance was read at 532 nm.

**Dose reduction factor (DRF):** The protective capacity of an agent (chemical or plant extract) is expressed as dose reduction factor (DRF). It can be calculated by dividing the LD<sub>50/30</sub> of *Rajgira* + irradiation by LD<sub>50/30</sub> of irradiation alone animals. **Irradiation alone Group (Irradiation alone):** These animals were exposed to 6, 8 and 10 Gy of Gamma rays and observed for 30 days to record the mortality and signs of radiation sickness. **Experimental Group (RE + Irradiation):** Animals of this group were given RE orally at the dose level of 800 mg/kg body weight/day for 15 consecutive days and exposed to 6, 8, and 10 Gy of gamma rays after the last administration. The animals were observed for 30 days and radiation sickness as well as mortality was recorded in similar manner as it was recorded in irradiation alone group.

**Modification of radiation response:** The animals selected for this study were divided into two groups. Animals of one

group (experimental) were administered *Rajgira* extract orally (800mg/kg body weight/day for 15 consecutive days), and the irradiation alone group received DDW (volume equal to RE). After 30 minutes of last treatment all the animals were exposed to different doses of gamma radiation (6, 8 and 10 Gy).

**Body weight:** The general condition and body weights of the mice in all groups were observed daily. The per cent change in body-weight in each group of mice was recorded every day by dividing the average body weight of those mice on the first day of treatment as described elsewhere.<sup>43)</sup>

### Endogenous spleen colony assay

The endogenous spleen colony assay was done according to method of Till and McCulloch.<sup>25)</sup> Endogenous spleen colony forming units (CFU-S) were determined on day 10 after irradiation. Animals were sacrificed by cervical dislocation; their spleens were removed, weighed and fixed in Bouins fixative. Grossly visible nodules on the surface of the spleen were counted with naked eye.

### Survival assay

Mice of both groups (irradiation alone as well as experimental) exposed to 6, 8 and 10 Gy gamma radiation were checked daily for 30 days and the percentage of mice surviving 30 days of exposure against each radiation dose was used to construct survival-dose response curves.

### Quantitative changes in spleen

The weight of spleen at each autopsy interval (day 1, 3, 7, 10, 14 and 30 post irradiation) was determined to study the changes.

### Statistical analysis

The results obtained were expressed as mean  $\pm$  SE. Student's 't' test was used to make a statistical comparison between the groups. Significance levels were set at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ . Regression analysis was done to obtain LD<sub>50/30</sub> values and to determine dose reduction factor (DRF).

## RESULTS

Treatment with *Rajgira* leaf extract for 15 consecutive days in mice did not produce any toxic effect. Rather, these animals showed an increase in body weight at 30 days as compared to sham irradiated animals. A significant decrease in GSH content was observed in irradiation alone animals (Irradiation alone) whereas, *Rajgira* + irradiation showed a significant increase in GSH content (blood as well as liver) at various concentrations of *Rajgira* extract. However, maximum increase in GSH content was observed in the animals pretreated with 800 mg/kg body weight/day RE and irradiated (Table 1). An increase in TBARS level in liver and serum was also evident in irradiation alone animals.

**Table 1.** Reduced glutathione (GSH) and Lipid peroxidation (LPO) levels in blood and liver of Swiss albino mice with or without *Rajgira* extract (RE) treatment and/or exposed to radiation (8 Gy).

Treatment	GSH		LPO	
	Blood (µg/ml)	Liver (µmole/gm)	Blood (nmole/ml)	Liver (nmole/mg)
Sham irradiated (Normal)	1.85 ± 0.004	58.97 ± 1.44	1.46 ± 0.092	2.89 ± 0.11
<b>RE alone</b>				
200 mg	1.85 ± 0.006	59.13 ± 0.68	1.43 ± 0.049	2.86 ± 0.067
400 mg	1.86 ± 0.003	59.35 ± 0.22	1.39 ± 0.10	2.63 ± 0.092
800 mg	1.88 ± 0.007 <sup>b</sup>	60.23 ± 0.42	1.31 ± 0.092	2.34 ± 0.093 <sup>b</sup>
1200 mg	1.87 ± 0.004 <sup>b</sup>	59.96 ± 0.28	1.28 ± 0.074	2.36 ± 0.011 <sup>b</sup>
Radiation alone (8 Gy)	0.779 ± 0.013 <sup>c</sup>	34.34 ± 0.28 <sup>c</sup>	4.46 ± 0.066 <sup>c</sup>	7.71 ± 0.075 <sup>c</sup>
<b>RE + Radiation</b>				
200 mg + 8 Gy	0.866 ± 0.006 <sup>c</sup>	36.97 ± 0.39 <sup>c</sup>	4.11 ± 0.076 <sup>c</sup>	7.33 ± 0.077 <sup>b</sup>
400 mg + 8 Gy	0.935 ± 0.012 <sup>c</sup>	42.53 ± 0.21 <sup>c</sup>	3.41 ± 0.087 <sup>c</sup>	6.77 ± 0.068 <sup>c</sup>
800 mg + 8 Gy	1.327 ± 0.004 <sup>c</sup>	48.19 ± 0.36 <sup>c</sup>	3.12 ± 0.068 <sup>c</sup>	4.61 ± 0.067 <sup>c</sup>
1200 mg + 8 Gy	1.318 ± 0.005 <sup>c</sup>	48.02 ± 0.24 <sup>c</sup>	3.15 ± 0.068 <sup>c</sup>	4.44 ± 0.066 <sup>c</sup>

Sham irradiated = No treatment **Significance level** : a -p < 0.05, b- P < 0.01, c- P < 0.001

Radiation alone = 8 Gy gamma radiation **Statistical Comparison**: Radiation alone V/s sham irradiated

RE alone: mg/kg body weight RE was given orally for 15 consecutive days

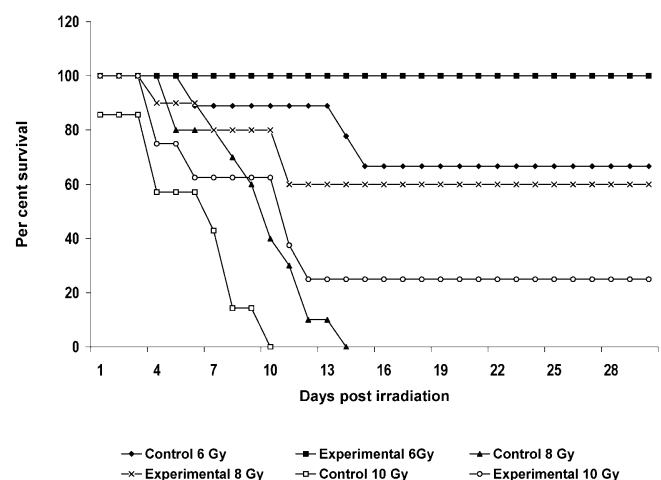
RE + Radiation V/s Radiation alone

RE alone V/s sham irradiated

Although, no significant difference was noticed in such levels in sham irradiated and RE treated animals. But, a significant dose dependent decrease was registered in RE pretreated irradiated animals. However, the maximum decline in LPO level was measured in the animals pretreated with 800 mg/kg body weight/day RE (Table 1). Therefore, 800 mg/kg body weight/day RE was used for detailed study.

In the present study, it was observed that pretreatment of RE enhanced the survival of mice exposed to different doses of gamma radiation. The severity of the radiation sickness was dose dependent and 34% of the animals died within 30 days post irradiation with 6 Gy, whereas, 100% mortality was observed on day 14 and 10 in animals of irradiation alone groups after exposure to 8 and 10 Gy respectively. The survivability in 6 Gy experimental group was 100% but it decreased to 66 and 25% in experimental groups after irradiation with 8 and 10 Gy respectively (Fig. 1). Regression analysis of survival data showed 6.33 and 8.62 Gy LD<sub>50/30</sub> values for irradiation alone and *Rajgira* + irradiation producing a DRF as 1.36.

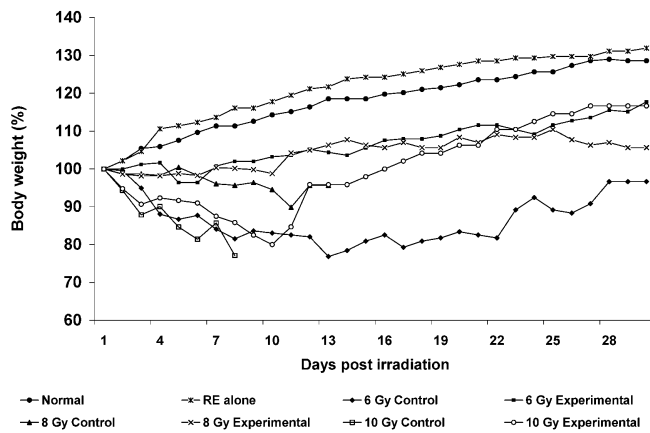
Maximum body weight loss was 24% and minimum loss was 13.5% in irradiation alone groups whereas, in experimental groups it was 22.05 and 1.7% in their respective groups. Not only this, but the *Rajgira* + irradiation showed 17% (6 Gy), 9.5% (8 Gy) and 13.7% (10 Gy) increase in



**Fig. 1.** Per cent survival of Swiss albino mice after exposure to 6, 8 and 10 Gy gamma rays with or without pretreatment of *Rajgira* extract.

their body weight than the initial ones at day 30 post-irradiation (Fig. 2).

It was observed that RE pretreatment to mice increased the number of spleen colonies significantly than the irradiation alone group (Table 2). The pattern of spleen weight change was similar in all the irradiation alone groups upto



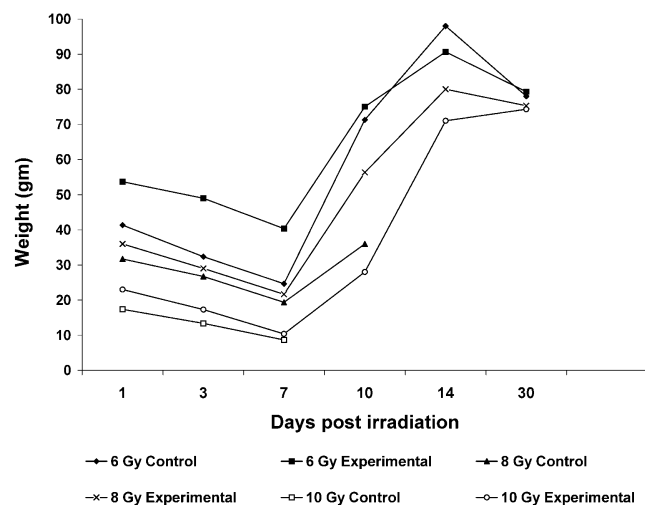
**Fig. 2.** Per cent change in body weight of Swiss albino mice with or without pretreatment of *Rajgira* extract (RE) and exposed to 6, 8 and 10 Gy gamma radiation.

**Table 2.** Number of grossly visible colonies on spleen on day 10 after single total body irradiation to different doses of gamma radiation with or without *Rajgira* extract (RE) treatment.

Treatment	Number of spleen colonies
Sham irradiated (Normal)	–
Irradiation alone (6 Gy)	1.33 ± 0.72
RE + 6 Gy	20.67 ± 0.72
Irradiation alone (8 Gy)	–
RE + 8 Gy	15.33 ± 0.98
Irradiation alone (10 Gy)	–
RE + 10 Gy	10.0 ± 0.47

Normal = No treatment

RE = 800 mg/kg body weight RE



**Fig. 3.** Changes in spleen weight of Swiss albino mice exposed to 6, 8 and 10 Gy gamma rays with or without pretreatment of *Rajgira* extract.

day 7 after irradiation, but the decrease in spleen weight was found to be dose dependent, i.e. higher the radiation dose, more the weight loss. The maximum weight loss was observed at day 7, after that increase in the tissue weight was registered. The spleen weight in RE treated and *Rajgira* + irradiated animals decreased till day 7 but the decrease was significantly less as compared to irradiation alone group at each autopsy interval. After day 7 a gradual increase was observed which attained almost normal value by day 30 (Fig. 3).

## DISCUSSION

The results of the present study indicate that pretreatment of *Rajgira* extract (RE) protect the hematopoietic tissues in mice from the lethal effects of ionizing radiation. The radioprotective effect of RE was demonstrated by determining the LD<sub>50/30</sub> values (DRF=1.36) and endogenous spleen colony assay. A significant radioprotection was observed when 800 mg/kg body weight/day RE was given orally for 15 consecutive days before radiation exposure.

The free radicals generated during the radiolysis of water play the most important role in the direct biological damage induced by ionizing radiation.<sup>26)</sup> Under normal conditions, the inherent defense system including glutathione and antioxidant enzymes protects against the oxidative damage. GSH offers protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation.<sup>27)</sup> GSH is a versatile protector and executes its radioprotective function through free radical scavenging, restoration of the damaged molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state.<sup>28)</sup> A significant decrease in GSH content in liver and blood was observed following gamma irradiation (8 Gy). The oral administration of *Rajgira* extract (RE) did not influence the endogenous GSH content significantly, but it protects the GSH depletion due to irradiation. These results suggest that endogenous non-protein sulfhydryl content (GSH) is maintained by the extract in these *Rajgira* + irradiation group. The oxidative stress due to the radiation induced free radicals can cause a dramatic fall in the hepatic GSH, which overwhelms the cellular defense and lead to membrane lipid peroxidation and a loss of protective thiols.<sup>29)</sup> GSH might be reacting with the peroxide intermediates; since peroxide intermediates stimulate further lipid peroxidation by autocatalysis and enhance the damage.

The basic effect of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. Lipid peroxidation can be initiated by radiolytic products, including hydroxyl and hydroperoxyl radicals.<sup>30)</sup> In the present study, it was observed that, although *Rajgira* extract treatment did not significantly alter the lipid peroxidation level in unirradiated animals but *Rajgira* extract pretreatment significantly lowers the radiation induced lipid peroxidation in

terms of malondialdehyde production in a dose dependent manner. Inhibition of lipid peroxidation in bio membranes can be caused by antioxidants.<sup>31-32</sup> It has been demonstrated that both  $\alpha$ -tocopherol and  $\beta$ -carotene react directly with peroxy radicals involved in lipid peroxidation *in vitro*. When used in combination, these antioxidants have an additive effect in preventing peroxidation of microsomal lipids in solution.  $\beta$ -carotene may protect the endogenous  $\alpha$ -tocopherol present in microsomal lipids, thus having a dual function.<sup>33</sup>

Mortality occurred after irradiation, in addition to GI and hematopoietic syndromes, is being attributed to inhibition of the immune system, i.e. irradiation causes immuno-suppression leading to death of the animal. Endogenous infections too might have contributed to the death of the irradiated mice. Bacteremia may be a cause of mortality secondary to haematopoietic and gastrointestinal radiation damage.<sup>35</sup> The maximum mortality was occurred in all the groups within eight days post-irradiation. These findings suggest that the injuries to GI as well as haematopoietic organs were responsible for causing the death in the used dose range. Samarth *et al*<sup>34</sup> have reported that *Mentha* extract pretreatment provides protection against radiation induced alterations in intestinal mucosa of Swiss albino mice. *Mentha* pretreatment resulted in significant increase in villus height, number of total cells and mitotic cells, whereas goblet cells and dead cells showed a significant decrease as compared to irradiated irradiation alone (8 Gy).

The pattern of changes in body weight following whole-body irradiation is in good agreement with those reported earlier.<sup>36</sup> In irradiation alone animals of this study, decrease in body weight was observed between first two weeks (depending on radiation dose), which may be attributed to reduced food and water intake, loss of fluid and electrolytes through diarrhoea and diminished absorption capacity of the GI tract. The RE pretreated irradiated animals showed less change in body weight as compared to irradiation alone and recovery begins from day 7, 10 and 17 after exposure to 6, 8 and 10 Gy respectively. Similarly, Rugh and Wohlformm<sup>37</sup> also reported reduction in body weight due to radiation induced cell death after exposure and was found to be dose-dependent in mice.<sup>38-39</sup>

It was evident that RE pretreatment elevated the number of radiation induced endogenous spleen colonies and increased spleen weight respectively. The spleen weight on day 14 was recorded above normal at 6 Gy, which indicates a compensatory action for the aplastic bone marrow.<sup>40</sup> Animals pretreated with *Rajgira* extract and irradiated to different doses of radiation exhibited less degree of weight reduction in spleen as compared to their respective irradiation alones. The recovery was also earlier and the spleen weight attained almost normal value by day 30. The similar results were observed during histopathological study of spleen as *Rajgira* + irradiation showed an early recovery as compared

to their respective irradiation alone groups.<sup>41</sup> It has been reported that aqueous extract of *Rajgira* protects Swiss albino mice against gamma radiation, and significantly higher values of blood corpuscles (RBC/WBC), hematocrit percentage (Hct) and hemoglobin (Hb) level were observed in *Rajgira* extract pretreated irradiated animals as compared to irradiated irradiation alone animals.<sup>42</sup> An earlier study by Samarth and Kumar<sup>43</sup> in our laboratory, showed that aqueous extract of *Mentha* protects Swiss albino mice against gamma radiation, and observed significantly higher values of blood corpuscles; hematocrit percentage and hemoglobin level in *Mentha* pretreated irradiated animals as compared to irradiated alone animals. Further, a significant enhancement in the number of endogenous spleen colonies has been observed in *Mentha* pretreated irradiated animals.<sup>43</sup> These results suggest that one of the mechanisms of radioprotection offered by extract is due to stimulation/protection of hematopoietic system.

Thus the results of the present study suggest that RE pretreatment provides protection against radiation induced sickness, body weight and spleen weight loss, mortality and it also maintains GSH and LPO levels in blood and liver. The hematopoietic stem cells can be protected from radiation induced free radical damage by RE, which was evident in the increased number of spleen colonies (CFU-S) in animals pretreated with *Rajgira* extract. It has been reported that plant flavonoids that show antioxidant activity *in vitro* also function as antioxidants as *in vivo*, and their radioprotective effect may be attributed to their radical scavenging activity.<sup>44</sup> The radioprotective effect of *Rajgira* extract (RE) may be assigned to its antioxidant properties as it contains provitamin A ( $\beta$ -carotene), vitamin C and riboflavin.<sup>12</sup>

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