

Protective Effect of *Adhatoda vasica* leaf extract on body weight and spleen weight of gamma-irradiated mice

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Abstract: Effect of ethanolic extract of *Adhatoda vasica* against gamma radiation induced changes in terms of body weight and spleen weight in Swiss albino mice was studied at post-irradiation intervals between 1 and 30 days. Mice exposed to 6 Gy gamma radiation showed radiation induced sickness including changes in body weight and spleen weight of mice. When ethanolic leaf extract of *Adhatoda vasica* was given orally at a dose of 900 mg/kg body weight prior to irradiation showed a significant protection. Mice were divided into four groups i.e. Group I containing normal mice served as control for each experimental groups; group II mice given 900 mg/kg body wt. of *Adhatoda vasica* extract orally for 15 days; group III mice were exposed to gamma radiation (6Gy) and group IV mice given *Adhatoda vasica* extract for 15 days then exposed to gamma radiation (6 Gy). There was significantly lesser degree of reduction in body weight and spleen weight to extract treated plus irradiated mice. But there was higher degree of reduction in irradiated mice body weight and spleen weight.

Keywords: *Adhatoda vasica*, gamma radiation, body weight, spleen and oral administration.

1. INTRODUCTION

Several biological effects of plants and plant extracts were investigated in recent clinical and pharmacological studies, thus confirming its efficacy in Indian system of medicines. Many more medicinally useful plants are present in Himalayas. Among them are *Adhatoda vasica* used in the present study belongs to the family Acanthaceae and is found throughout India upto the height of 1300 m. In the ayurvedic nomenclature it is referred as Vasaka or Vrisha while in general it is called Basuti or Bansa. The leaves of plant are the main source of drug preparation. Extracts of various plants have been reported to be beneficial for free radical mediated conditions in humans, as they contain compounds having antioxidant activity which can prevent damage induced by reactive oxygen species. The plant has been used in the indigenous system of medicine worldwide as herbal remedy for treating cold, cough, chronic bronchitis, asthma, as sedative expectorant, rheumatism and rheumatic painful inflammatory swellings. Plants provide complicated, mixed and distinct non-nutrient elements which act as the main basis of drug discovery [1]. Major symptoms of radiation sickness are reduced food and water intake, weight loss, diarrhea, lethargy, hair loss, disorientation, epilation and necrosis in the tail. Ionizing radiation at lower doses causes hematopoietic syndrome and at higher dose causes gastrointestinal syndrome and central nervous syndrome. Interaction of ionizing radiation with living cells cause a variety of changes, whose damage intensity depend fundamentally on the absorbed dose, type of radiation, conditions of irradiation and intrinsic radiosensitivity of cell [2]. Plants products appear to have an advantage over the synthetic compounds in terms of low/ no toxicity at the effective dose with minimum or no side effects [3]-[7]. The spleen is a dark red to blue-black organ located in the left cranial abdomen. The functions of the spleen are centered on the systematic circulation. It is composed of two functionally and morphologically distinct compartments, the red pulp and the white pulp. The red pulp is a blood filter that remove foreign materials and damaged erythrocytes. It is also a storage site for iron, erythrocytes and platelets. Spleen is also the largest secondary lymphoid organ containing about one-fourth of body's lymphocytes and initiates immune responses to blood borne antigens [8]-[10].

2. MATERIALS AND METHODS

Swiss albino mice of Balb-C strain weighing 22-25g were procured from Central Research Institute (CRI) Kasauli, Himachal Pradesh, India. These were maintained in the animal house of Department of Biosciences of Himachal Pradesh University, Shimla under proper hygienic conditions (24±2 C temp. and light). Mice were provided Hindustan lever feed and water *ad libitum*. The entire animal care and experimental procedures were approved by the Institutional animal ethics committee of Himachal Pradesh University, Shimla (IAEC/Bio/12-2009).

Plant material:

Leaves of *Adhatoda vasica* were collected from herbal garden Joginder Nagar, Himachal Pradesh, India.

Extraction of plant materials:

Dried leaves powder was extracted five times with 80% ethanolic solution. Extraction was done after every twenty four hours. Collected suspension was concentrated under reduced pressure.

Source of Irradiation:

About 6-8 weeks old male mice were irradiated in "Gamma chamber-900" (BARC) with automatic timer having cobalt-60 as the source of gamma rays.

Experimental design:

Normal healthy animals showing no sign of morbidity were divided into following groups: i) Mice in first group serve as control ii) Mice of second group were administered *Adhatoda vasica* extract (900 mg/kg body wt.) for 15 days. iii) Mice of third group were exposed to gamma radiation (6 Gy). iv) Mice of fourth group were administered *Adhatoda vasica* extract (900 mg/kg body wt.) for 15 days and then exposed to gamma radiation (6 Gy).

Extract and Radiation Administration

The mice were given *Adhatoda vasica* extract orally (900 mg/kg body wt.) for 15 days and after 30 min. of last dose; they were exposed to 6 Gy dose of gamma radiation. Following various treatments, mice were autopsied by cervical dislocation on day 1, 5, 15 and 30. The spleen of normal, extract treated, irradiated and extract treated plus irradiated mice was excised.

3. RESULTS

The results obtained for body weight and spleen weight of mice were presented in figures I & II and Tables I & II as follows:

Body weight (Table I & Fig. I).

A record of body weight of normal, *Adhatoda vasica* extract treated, irradiated and *Adhatoda vasica* extract treated plus irradiated mice was maintained during the investigation period at each stage. The body weight of normal mice was 22.20 ± 0.46 g at day 1, 22.65 ± 0.36 g at day 5 and 23.81 ± 0.31 g at 15 days stage. Finally the body weight was found to be 25.10 ± 0.37 g at 30 days stage. A slight increase in body weight of control mice was observed throughout the period of study.

The dose of *Adhatoda vasica* extract (900 mg/kg body weight) led to slight increase in body weight of mice. The body weight of mice on 1 day stage was 22.40 ± 0.36 g after extract treatment and percent increase was 0.9%. On day 5, the weight was 22.86 ± 0.23 g showing increase of 0.92%. On day 15, the weight was 23.51 ± 0.63 g and percent rise was 1.26%. The weight of the mice at 30 day was 26.61 ± 0.35 g and percent increase was 5.68%.

Irradiated mice showed decrease in body weight. The weight of mice at 1, 5 and 15 days stages was 21.31 ± 0.49 g, 20.70 ± 0.24 g and 19.78 ± 0.48 g after radiation treatment thereby leading to decrease of 4.00%, 9.42% and 20.37% in comparison to normal mice. At 30 days stage, no survival of mice was seen.

Adhatoda vasica extract plus irradiated mice showed significant increase in body weight. The weight of mice at 1, 5 and 15 day stage was 22.18 ± 0.55 g, 21.71 ± 0.43 g, 23.48 ± 0.62 g after *Adhatoda vasica* extract treatment plus irradiation thereby leading in increase of 3.93%, 4.66% and 15.76% in comparison to irradiation. The weight of mice at 30 day was 25.23 ± 0.41 g and percentage increase was not recorded at this stage because no mice survived in irradiated group upto 30 days stage.

Table I

Groups	Days			
	1	5	15	30
N	22.20 ± 0.46	22.65 ± 0.36	23.81 ± 0.31	25.10 ± 0.37
ET	22.40 ± 0.36	22.86 ± 0.23	23.51 ± 0.63	26.61 ± 0.35*
IR	21.31 ± 0.49	20.70 ± 0.24**	19.78 ± 0.48**	NS
ET+IR	22.18 ± 0.55	21.71 ± 0.43	23.48 ± 0.62**	25.23 ± 0.41

N = Normal; ET = Extract treated; IR = Irradiated; ET+IR = Extract treated + Irradiated; NS = No survival

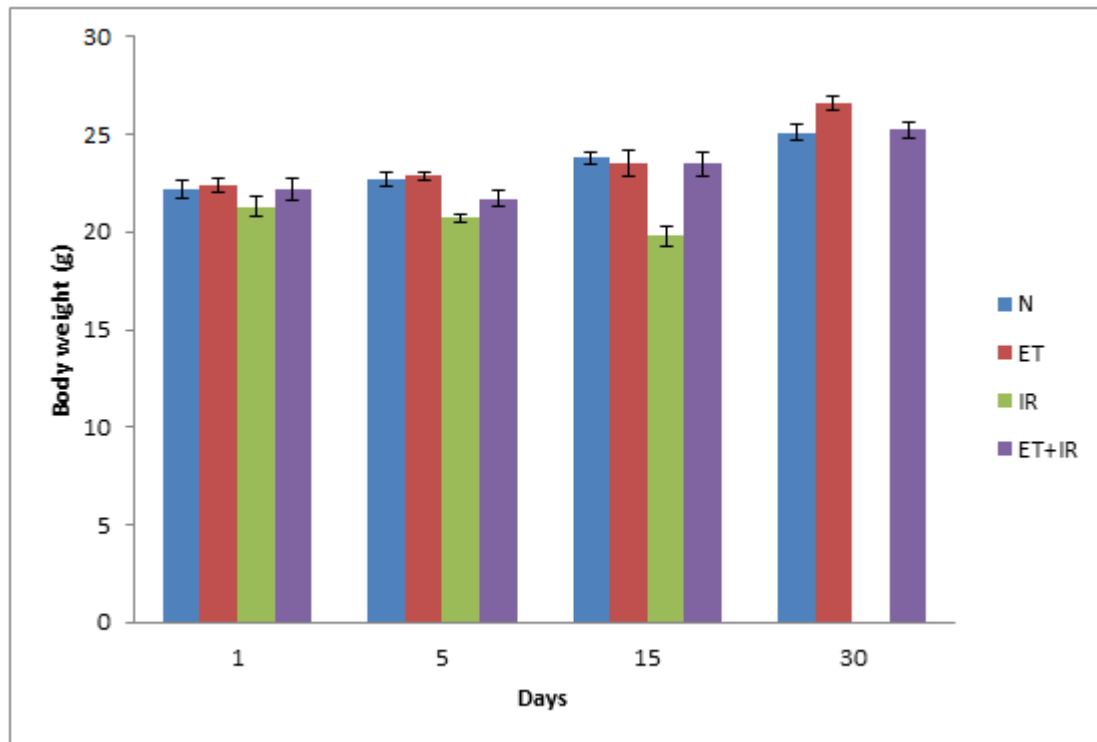


Fig. I

Table I & Fig. I: Changes in body weight (g) of normal, extract treated, irradiated and extract treated plus irradiated mice from 1-30 days period. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).

Spleen weight (Table II & Fig. II).

The weight of spleen of the normal mice was recorded as 99.00 ± 0.36 mg, 99.66 ± 0.55 mg, 100.66 ± 0.61 mg and 101.50 ± 0.50 mg at 1, 5, 15 and 30 days stages respectively.

The spleen weight of *Adhatoda vasica* extract treated mice showed significant increase, which was recorded as 99.83 ± 0.94 mg, 100.83 ± 1.07 mg, 101.66 ± 0.76 mg and 102.66 ± 0.33 mg at 1, 5, 15 and 30 days and percent increase of 0.84%, 1.17%, 0.99% and 1.13% respectively in comparison to normal mice.

Irradiated spleen weight was noticed as 97.66 ± 0.66 mg, 96.83 ± 0.60 mg and 95.16 ± 0.60 mg at 1, 5 and 15 days with percentage decrease of 2.22%, 4.13% and 6.83 % respectively as compared to *Adhatoda vasica* extract treated mice. At 30 days stage no survival of mice was seen in irradiated group so comparison could not be made.

Adhatoda vasica extract treated plus irradiated mice spleen weight was recorded as 98.00 ± 0.51 mg, 97.66 ± 0.49 mg, 96.66 ± 0.71 mg and 98.00 ± 0.36 mg at 1, 5, 15 and 30 days with percent increase of 0.35%, 0.85% and 1.56% respectively as compared to irradiated mice. At 30 days stage percentage increase was not observed as no mice survived upto 30 days in irradiated group.

Table II

Groups	Days			
	1	5	15	30
N	99.00 ± 0.36	99.66 ± 0.55	100.66 ± 0.61	101.50 ± 0.50
ET	99.83 ± 0.94	100.83 ± 1.07	101.66 ± 0.76	102.66 ± 0.33
IR	97.66 ± 0.66	96.83 ± 0.60**	95.16 ± 0.60**	NS
ET+IR	98.00 ± 0.51	97.66 ± 0.49*	96.66 ± 0.71**	98.00 ± 0.36**

N = Normal; ET = Extract treated; IR = Irradiated; ET+IR = Extract treated + Irradiated; NS = No survival

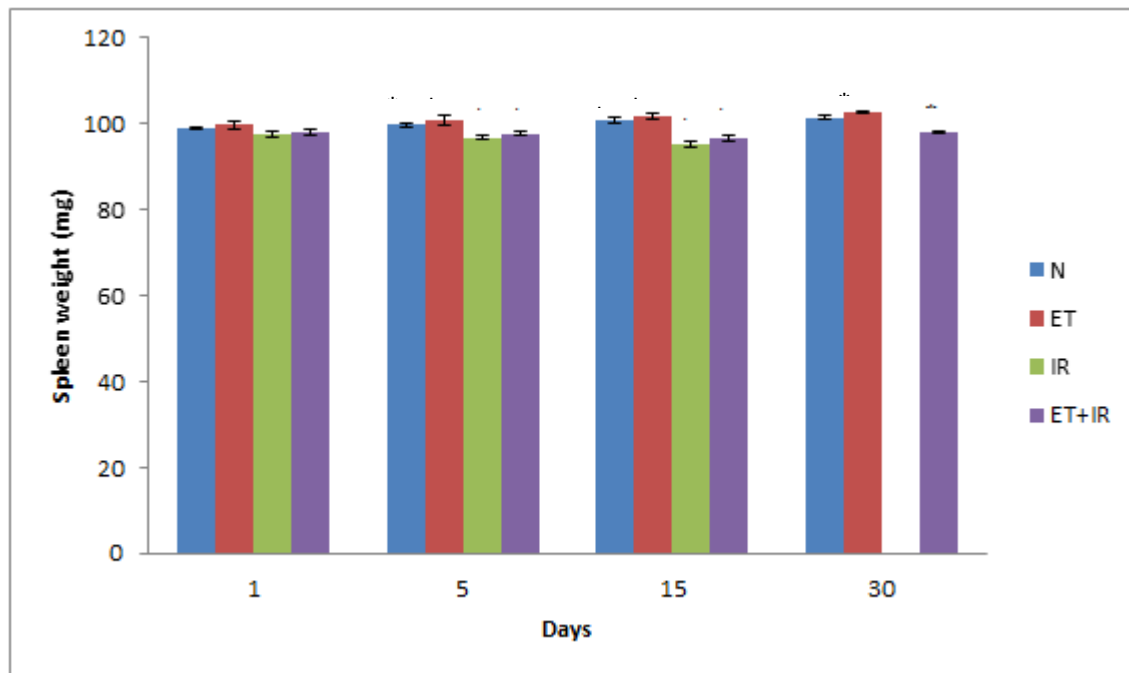


Fig. II

Table & Fig. II: Changes in spleen weight (mg) of normal, extract treated, irradiated and extract treated plus irradiated mice from 1-30 days period. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).

4. DISCUSSION

The present study has been convincingly demonstrated that *Adhatoda vasica* extract treatment results significant change in body weight of mice. However, on days 30 a significant weight increase was observed as compared to mice of control. Since change in body weight has been used as an indicator of good health [11], [12]. The present study suggested that dose of 900 mg/kg body wt. of *Adhatoda vasica* extract administered orally is non-toxic to mice. Body weight of irradiated mice exhibited a gradual declining pattern. The weight loss may be due to the gastro-intestinal damage following irradiation. The weight loss is also associated with decrease in water intake by animals [13]. In present study after exposure to 6 Gy gamma radiations to animals resulted in radiation sickness within 3-5 days after exposure. The symptoms included reduction in the food and water intake, weight loss, diarrhoea, ruffling of hairs and irritability. These symptoms have been also observed in mice after gamma irradiation by [14]. *Adhatoda vasica* extract treated plus irradiated mice showed significant increase in body weight in comparison to irradiated mice. It is evident from present study that administration of *Adhatoda vasica* extract reduced radiation sickness and mortality and provided protection to gastro-intestinal tract from the damaging gamma radiation. It is observed that this extract is effective in circulation.

Analysis of spleen weight is an important endpoint for identification of potentially harmful effects of chemicals in toxicological studies [15]. *Adhatoda vasica* extract treated mice showed significant gain in spleen weight. A notable recovery in body weight was ensued from day 5 onward in extract treated plus irradiated animals but without achieving the normal weight even till the end of experiment. These results suggest the possibility of protection of gastrointestinal system by *Adhatoda vasica* since radiation induced loss in body weight is due to the decrease in food and water intake due

to gastrointestinal damage as also described by [16]. Some earlier studies also showed that administration of crude extracts of different herbal drugs reduced radiation induced loss in body weight of animals [17]-[19]. Weight of irradiated mice spleen exhibited a gradual declining pattern. This finding is supported by decrease in hematological constituents may be due to a direct damage by radiation [20].

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