RADIATION-INDUCED HEMATOLOGICAL ALTERATIONS
AND THEIR INHIBITION BY AEGLE MARMELOS FRUIT EXTRACT

by

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This study was carried out to observe the radio protective potential of Aegle Marmelos fruit extract (AME) against radiation-induced hematological and biochemical alterations in blood and liver of mice. For this purpose, adult Swiss albino mice were exposed to 6 Gy gamma radiation in the presence (experimental) or absence (control) of the extract (100 mg/kg body weight animal/day). Exposure to radiation resulted in a significant decline in the count of erythrocyte, hemoglobin (Hb) and hematocrit (Hct) in peripheral blood. In contrast, extract-pretreated irradiated animals had a significant rise in all of these blood constituents, as compared with the irradiated control. Furthermore, a significant elevation in lipid peroxidation over normal was recorded in the irradiated control, whereas such increase was considerably lesser in extract-pretreated animals. Likewise, pretreatment with AME caused a significant increase in glutathione levels in the serum, as well as in the liver, in comparison to irradiated controls. These results indicate that AME may be responsible for the protection of stem cells in bone marrow, subsequently resulting in a rise of hematological constituents in peripheral blood. The present study affirms the prophylactic use of AME against radiation-induced hematological and biochemical alterations in mammals.

Key words: gamma radiation, glutathione, hematological constituents, lipid peroxidation, Aegle Marmelos, Swiss albino mice

INTRODUCTION

Radiation is the most studied environmental hazard in the world. Ionizing radiations including alpha, beta, and gamma rays and neutrons with sufficient energy to generate ion pairs, i.e., electrons which can generate chemically active free radicals can, in turn, damage the molecular structure, resulting in cellular dysfunctions or mutations [1]. The inadvertent exposure of humans to various sources of radiation causes the ionization of molecules, setting off potentially damaging reactions, via free radicals production. Free radicals are believed to play a role in more than sixty different health conditions, including the ageing process, cancer, radiation damage, atherosclerosis etc. [2, 3].

In today’s highly nuclear threatened environment, there is an increased need to protect not only high risk service groups from the hazards of unintended ionizing radiation exposure, but the general public, as well. Thus, there is an urgent need for the development of radioprotective agents. Fortunately, there are many plant derived natural antioxidants that interfere with free radicals before they can damage the body. Antioxidants work in several ways, either by reducing the energy of the free radicals, stopping the free radicals from forming in the first place, or interrupting an oxidizing chain reaction and, thus, minimizing the damage this causes.

Radiation damage results from the sensitivity of cells to radiation, with those that replicate most rapidly being those most sensitive to radiation exposure. Mature cells that are more highly differentiated appear to be least affected by radiation. This difference in cell sensitivity is the basis for the distinction among the three sub-syndromes of the acute radiation syndrome (ARS). ARS is divided into hematopoietic, gastro-intestinal, and neurovascular sub-syndromes. Human beings overexposed to radiations are prone to developing life-threatening diseases, often related to the hematopoietic system. This being the result of the fact that the hematopoietic system is highly sensitive to ra-
radiation and that peripheral blood counts may well serve as a biological indicator of such damage. The target cells of the hematopoietic tissues are the stem cells. Radiation protection concepts and philosophy have been evolving over the past several decades. Several synthetic compounds have been used in the past as radio protectors. For the first time, Patt et al., observed that the pretreatment of rats and mice with cysteine (naturally occurring amino acid) before exposure to radiation protected them against radiation-induced sickness and mortality [4]. Subsequently, several chemical compounds were synthesized and tested for their radioprotective ability [5]. However, the major drawback of these compounds has been their high toxicity at optimum doses. Therefore, there is a need to screen alternatives which are non-toxic or less toxic at their optimum protective doses for practical purposes.

Herbal drugs offer an alternative to synthetic compounds and have been considered either non-toxic or less toxic, thus giving an impetus to the screening of their radio protective properties. Aegle Marmelos, commonly known as bael, is a spinous tree belonging to the family Rutaceae. It is widely found in India, Bangladesh, Burma, and Sri Lanka. It is distributed mainly within the sub-Himalayan forests, in dry hilly regions. It is called Shivadune, the tree of lord Shiva. Aegle Marmelos plays an important role in the indigenous system of Indian medicine. Its edible leaf, root, bark, seed, and fruit are highly valued in Ayurvedic medicine in India [6]. In fact, since Charaka (1500 BC), no drug has been longer or better known or appreciated by the inhabitants of India than the bael [7]. The fruit of this plant has been used as an astringent, relief for indigestion, and cure for the treatment of diarrhea, dysentery, and stomachalgia. Aqueous AME exhibits an anti-hyperlipidaemic [8] and hypoglycemic [9] effect in ptozotocin-induced diabetic rats. The ripe fruit of this plant is used in different formulae for the treatment of chronic diarrhea [10].

Envisioning a possible use of the pharmacological and therapeutic values of this plant, the present study has been carried out in order to access the protective potential of the fruit of Aegle Marmelos against radiation-induced hematological and biochemical alterations in mammals.

MATERIALS AND METHODS

Animal care and handling

Animal care and handling were carried out according to the guidelines set by the World Health Organization, Geneva, and the Indian National Science Academy (INSA), New Delhi. Male Swiss albino mice (Mus Musculus), 6 to 8 weeks old and weighing 20-24 g, from an inbred colony, were used for the present study. These animals were kept under controlled conditions of temperature and light (light: dark, 10-14 hours). They were provided with standard mice feed (procured from Aashirwad Industries, Chandigarh, India) and water ad libitum. As a preventive measure against infections, tetracycline water was given once a fortnight. This study has the approval of the Departmental Animal Ethical Committee.

Irradiation

The Cobalt Teletherapy Unit (ATC-C9) at the Cancer Treatment Centre, Department of Radiotherapy, SMS Medical College & Hospital, Jaipur, India, was used for irradiation. Unanesthetized mice were restrained in a well ventilated Perspex box and whole-body exposed to 6 Gy gamma radiation.

Preparation of Aegle Marmelos extract

The fruits of Aegle Marmelos L. were collected locally after their proper identification by a competent botanist (Voucher Specimen no: RUBL-20438) from the herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India. The pulp was removed from the fruit and shade dried, powdered into a mixture and its hydro-alcoholic extract then prepared by refluxing it with double-distilled water (DDW) and alcohol (3:1) for 36 hours (12 × 3 hours) at 40 °C. The liquid extract was cooled and concentrated by evaporating its liquid content. The prepared AME was stored at a low temperature until further use. The extract was re-dissolved in DDW prior to oral administration to mice.

EXPERIMENTAL DESIGN

Determination of the optimum dose of AME against irradiation

Different doses of AME were tested against 8 Gy gamma radiations in Swiss albino mice in order to find out the optimal dose of AME on the basis of the survival percentage of such animals after up to 30 days of irradiation.

Modification of radiation response

A total of 70 animals used in the experiment were assorted into 4 groups. Five animals in Group I were administered with DDW, volume equal to AME (100 mg/kg body weight animal/day) by oral gavage, to serve as normal (vehicle treated); five mice in Group II were administered AME orally, once a day, with a dose of 100 mg/kg body weight animal/day for 5 consecutive days. In Group III, DDW volume equal to AME was administered for 5 consecutive days (as in
Group I). One hour after the last administration of DDW, such animals \( (n = 30) \) were exposed to 6 Gy gamma rays. Group IV mice \( (n = 30) \) were treated with AME orally for 5 consecutive days (as in Group II) and exposed to gamma radiation 1 hour after the last administration of AME on the 5th day.

All animals were observed daily for any signs of sickness, morbidity, behavioral toxicity, mortality and abnormality, if any. A minimum of 5 animals from each group were necropsied at 12 hours on days 1st, 3rd, 7th, 15th, and 30th post-treatment, in order to evaluate hematological and biochemical alterations.

**Hematological study**

Blood was collected from the orbital sinuses of animals from each group in a vial containing 0.5 M EDTA (ethylene diamine tetra acetate acid). Total numbers of erythrocyte (RBC), hematocrit (Hct), and hemoglobin (Hb) content were determined using standard procedures.

**Biochemical study**

**Lipid peroxidation (LPO) assay:** The lipid peroxidation (LPO) level in liver and blood serum was measured in terms of thiobarbituric acid reactive substances (TBARS) after 24 hours, using the method of Okhawa et al. [11]. The absorbance was read at 532 nm, using a UV-VIS Systronics spectrophotometer.

**Glutathione assay:** The hepatic level of reduced glutathione (GSH) was determined after 24 hours by the Moron et al. [12] method. The GSH content in blood was measured spectrophotometrically, using Ellman’s reagent with 5-5, dithiobis-2-nitrobenzoic acid (DTNB) as a coloring reagent, according to the method of Beutler et al. [13]. The absorbance was read at 412 nm.

**Statistical analysis**

The results for all groups at various necropsy intervals were expressed as a mean ± standard error (S. E.). Statistical differences between various groups were analyzed by the Student’s t test and their significance observed at different levels as \( p \leq 0.05, p \leq 0.01, \) and \( p \leq 0.001. \)

**RESULTS**

**Radiation sickness and mortality**

No toxic effects in terms of sickness were observed in the animals treated with DDW (Group I) and AME alone (Group II). Some of the animals exhibited signs of radiation sickness, such as anorexia, lethargy, diarrhea, body weight loss, and ruffled fur within 4 days after radiation exposure (Group III). No adverse effects were observed in terms of sickness, body weight, urination, defection pattern, and mortality in the irradiated animals (Group IV).

**Selection of the optimum dose of AME against irradiation**

The optimum dose of AME against lethal gamma radiation (i.e. 8 Gy) for Swiss albino mice was selected on the basis of the survival experiment, where a number of deaths and survivals were recorded for up to 30 days after irradiation. Mice treated with AME, at doses of 25, 50, 100, 200, 400, and 800 mg/kg body weight day for 5 consecutive days prior to irradiation, exhibited a 30%, 55%, 88%, 62%, 42%, and 33% rate of survival, respectively. The dose of 100 mg/kg body weight was found to be the optimum dose based on the above data and further studies were carried out using this dose of AME (fig. 1).

![Figure 1. 30 days survival of Swiss albino mice after exposure to 8.00 Gy gamma rays in the presence (experimental) or absence (irradiated control) of AME](image-url)
**Hematological study**

Animals treated with AME alone (Group II) did not show any significant changes in various hematological constituents (erythrocytes, Hb & Hct) in comparison with the normal (Group I). Throughout the experiment, erythrocytes showed a significant decrease from normal. The maximum decrease in the total erythrocyte count was scored on day 3rd, after which such cells increased in their number on day 7th, but the normal value could not be restored even at the last autopsy interval (i.e. day 30). A significant increase in the red cell count with respect to the irradiated control was noticed during the entire period of the study, returning to an almost normal value at the last autopsy interval (i.e. day 30; fig. 2).

Hemoglobin concentration in irradiated mice showed the maximum decrease on day 3rd. Later, a slight increase was observed from day 7th on, but the values remained below normal up to the last autopsy interval. Animals irradiated with pretreated AME exhibited a higher Hb concentration than Group III animals and values were found to be near normal by the end of the experiment (fig. 3).

Hematocrit percentage was found to be significantly lower in the irradiated control mice, with a maximum decline on day 3rd, recovery from day 7th on, and a return to near normal by the 30th day of irradiation. On the contrary, AME pretreated irradiated animals exhibited significantly higher hematocrit values throughout the experiment (fig. 4).

**Lipid peroxidation assay**

No significant difference in the levels of LPO in blood or liver was noticed between the sham-irradiated (Group I) and AME alone treated (Group II) animals. A significant increase in blood and hepatic LPO levels was noted in gamma-irradiated animals (Group III), as compared with normal animals. However, these levels were found to be significantly lower than in the AME-pretreated irradiated (Group IV) animals (fig. 5).

![Figure 2](image-url)  
**Figure 2.** Variations (mean ± S. E.) in erythrocyte count (10⁶/mm³) in peripheral blood of Swiss albino mice after exposure to gamma rays with (experimental) or without (irradiated control) of AME. Significance level: normal vs. irradiated control; irradiated control vs. experimental p values: (a) <0.05, (b) <0.01, (c) <0.001

![Figure 3](image-url)  
**Figure 3.** Variations (mean ± S. E.) in hemoglobin content (gm/dl) in peripheral blood of Swiss albino mice after exposure to gamma rays with (experimental) or without (irradiated control) of AME. Significance level: normal vs. irradiated control; irradiated control vs. experimental p values: (a) <0.05, (b) <0.01, (c) <0.001
Glutathione estimation

No significant alterations in GSH contents of either liver or blood were observed between normal and AME-treated animals. However, a statistically significant decrease in GSH was noted in the irradiated control (Group III) in comparison with Group I animals. The AME-pretreated irradiated (Group IV) animals exhibited a significant elevation in GSH, both in blood and liver, in comparison with Group III animals; however, the values remained below normal (fig. 6).

DISCUSSION

The mortality of animals after irradiation in the present study may be due to the hematopoietic syndrome. Such deaths can be correlated with the impairment of the immune system [14]. Endogenous infections may also be responsible for the deaths of irradiated mice. Bacteremia may be one of the causes of mortality secondary to hematopoietic and gastro-intestinal radiation damage, because antibiotic treatment has shown to increase the survival of mice irradiated in the LD$_{50/30}$ range [15, 16].

The radioprotective effect of AME has been demonstrated by the increased survival rate. Significant radioprotection was achieved when AME was given orally in doses of 100 mg/kg body weight day for 5 consecutive days prior to irradiation. In our experiment, a 12% mortality was evident at the said dose of AME. Similarly, plants such as *Emblica Officinalis* [17], *Rosemarinus Officinalis* [18] and *Alstonia Scholaris* [19] have also been reported to provide protection against radiation-induced sickness.

In the present study, signs of sickness and mortality were not observed in the sham-irradiated (Group-I) or AME alone treated (Group-II) animals. Within it, body weight loss in irradiated animals may be attributed to the reduced food and water intake, fluid loss by diarrhea and the diminished absorption capacity of the GI tract. In general, the present findings...
are in agreement with those of Jagetia et al. [20], and Chaudhary et al. [21], with the exception of doses of gamma radiation in mammals.

The hematopoietic system is one of the most radiosensitive systems and damage done to it may play a crucial role in the development of the hematopoietic syndrome, possibly resulting in death. The sensitivity of blood cells in man to radiation determines the order in which the drop in the respective counts occurs [22]. Radiation-induced hematological changes in peripheral blood have been extensively studied, owing to their physiological significance [23, 24].

In the present study, the reduction in blood components in the irradiated group may be attributed to the impairment of cell division, obliteration of blood-forming organs, alimentary tract injury [25], depletion of factors needed for erythropoiesis differentiation and reticulocyte release from the bone marrow [26], as well as to the loss of cells from the circulation by hemorrhage or leakage through capillary walls and/or the direct destruction of mature circulating cells [27]. The decrease in all these blood constituents is responsible for anemia. Maximum decline in erythrocytes, hemoglobin, and hematocrit was observed on the third day following irradiation, which is in agreement with the findings in earlier works [18, 28, 29].

AME pretreatment before irradiation significantly checked the radiation-induced decline in erythrocytes, Hb and Hct. AME may be responsible for a significant protection of erythropoietic cells in bone marrow, which is subsequently responsible for the increase in such hematological components. Bone marrow cells have been reported to be protected against radiation-induced damage by various other plant extracts, as well [20, 30, 31].

The products of lipid peroxidation such as malonaldehyde and 4-hydroxynonenal are toxic to living cells [16, 32]. In the present study, it has been observed that although AME treatment did not significantly alter the LPO level in non-irradiated animals, it significantly lowered the radiation-induced LPO in terms of malonaldehyde formation. Inhibition of LPO in biomembranes can be attributed to various antioxidants present in the extract of this plant. Similarly, other plant extracts containing antioxidants have been found conducive to checking the radiation-induced elevation of lipid peroxidation levels in mammal blood and liver [19, 21].

GSH is a versatile protector and executes its radioprotective function through free radical scavenging, restoration of damaged molecules by hydrogen donation, reduction of peroxides and maintenance of protein thiols in a reduced state [33]. A significant decline in GSH content from normal, in blood and liver both, was noticed after irradiation. This could be because of its enhanced utilization as an attempt to detoxify the free radicals generated by radiation. Oral administration of AME did not influence the endogenous GSH content significantly, but it protected against GSH depletion caused by irradiation. These results suggest that endogenous non-protein sulphydryl content (GSH) is maintained by the extract in the AME-irradiated group. Various plant products have been reported earlier as inhibitors of radiation-inducing the decline of GSH content in various tissues in mammals [17-19, 21].

Natural antioxidants exhibit a long window of protection, i.e., they provide some protection when administered hours before radiation exposure. Exogenous administration of antioxidants, such as glutathione, superoxide dismutase (SOD), antioxidant vitamins (A, C, and E), lipoic acid, as well as substances that mimic or induce the activity of endogenous antioxidant systems (e.g., selenium, zinc, copper salts, and metal complexes), have exhibited protective properties against hematopoietic syndrome death [34-37]. Several phytochemical constituents like aegelin, alloimperatorin, marmelide, marmeline, marmelosin, marmesin, psoralen, skimming, tannic acid, xanthotoxol and β-sitosterol are reported to be present in the Aegle
Marmelos fruit [38-40]. The phytochemicals present in this plant might be responsible for the reduction of radiation-induced lipid peroxidation and the protection of erythropoietic stem cells in bone marrow, subsequently resulting in the increased levels of various hematological components in the peripheral blood noted in the present study. Since significant protection is obtained with a non-toxic dose, AME may well have an advantage over known radioprotectors. Further research in order to determine the exact mechanism and clinical applicability of Aegle marmelos as a radioprotector is in progress at the moment.

CONCLUSION

Present results indicate that the use of Aegle Marmelos fruit extract possibly inhibits radiation-induced hematological alterations and oxidative stress during planned and unplanned radiation exposure in mammals.

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HEMATOLOGICAL ALTERATIONS INDUCED BY GAMMA RADIATION AND THEIR PROTECTION USING AEGLE MARMELOS

This study was conducted to evaluate the hematological alterations and their protection by Aegle Marmelos in mice exposed to gamma radiation. The study was carried out on adult white mice of 4-6 months of age with an average weight of 25-30 g. The mice were divided into two groups: the experimental group received a single application of 100 mg/kg of Aegle Marmelos extract on the day of radiation, while the control group received no treatment. The results showed a significant decrease in the count of red blood cells, hemoglobin, and hematocrit in the peripheral blood of the control group, whereas the experimental group showed a significant increase in these parameters compared to the control group. The levels of glutathione in the serum and liver of the experimental group were significantly higher than those in the control group. This study demonstrated the protective effect of Aegle Marmelos on gamma radiation-induced hematological alterations in mice.

Key words: gamma radiation, glutathione, hematological alterations, Aegle Marmelos, radioprotective effects.