

INFLUENCE OF SEEDS EXTRACT OF *TRIGONELLA FOENUM GRAECUM* (METHI) ON MICE EXPOSED TO GAMMA RADIATION

by

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The present study has been carried out to evaluate the radioprotective effect of *Trigonella foenum* seeds extract (TFE) on peripheral blood of mice. For this purpose, mice were orally given double distilled water (control) or optimum dose (100 mg/kg of body weight per day) of TFE for five consecutive days (experimental). Thirty minutes after the last administration of double distilled water or TFE, these were exposed whole-body to 5 Gy gamma radiation and autopsied between 12 hours to 30 days for hematological and biochemical estimation. Total erythrocyte count, hemoglobin level, and hematocrit percentage were decreased from normal in both the groups. A significant increase in these parameters was observed in TFE administered irradiated group, in contrast to without TFE irradiated one, by restoring towards normal values at the end of the experiment. From the results, it is evident that TFE may be responsible for the protection of stem cells in bone marrow which subsequently resulted in higher hematological constituents in peripheral blood. The study concludes the prophylactic use of such plant extract against radiation induced hematological alterations.

Key words: gamma radiation, hematological constituent, glutathione, lipid peroxidation, *trigonella foenum graecum*, swiss albino mouse

INTRODUCTION

Ionizing radiation can be defined as any type of electromagnetic or practical radiation with sufficient energy to ionize atoms or molecules; that is to eject electrons from their outer orbitals. Ionizing radiation passing through living tissues generates reactive free radicals which interact with critical macromolecules, such as DNA, proteins or membranes, and can induce cell damage and potentially, cell dysfunction and death. Damage to DNA may be the most important factor in cell death [1]. Exposure to high amounts of ionizing radiation results in damage to the haematopoietic, gastrointestinal or central nervous system, depending on radiation dose [2].

Ionizing radiations affect haematopoietic tissues and reduce the circulating blood cells which can result in septicaemia, haemorrhage, anaemia and death. One of the strategies for novel radioprotective agents is the stimulation, maintenance and proliferation of progenitor cells from bone marrow. Radioprotective agents

are synthetic compounds or natural products that are immediately administered before irradiation to reduce injuries caused by ionizing radiations. Several chemical compounds and their analogues have been screened for their radioprotective ability; however, their toxicity at optimum protective doses precluded their clinical use [3, 4]. Another major drawback of these compounds was that they were unable to provide post-irradiation protection. As a result of the side effect profile, narrow time windows of these agents necessitated the search for second generation drugs that are more effectively less toxic and with more acceptable properties with respect to route and frequency of administration.

Plant products have various pharmacological properties and have been used already for a long time for the treatment of various diseases. Hence, screening herbal drugs offers a major focus for new drug discovery. Fenugreek (*Trigonella foenum graecum*) is an annual herb that has a long history as both a culinary and medicinal plant. Fenugreek seeds are commonly used as spice in Indian homes. The plant has traditional use in diabetes and its antidiabetic potential has been experimentally evidenced [5]. Moreover, the plant is an

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excellent candidate for oral therapy as it is effective, non-toxic, and without serious side effects. The chemical constituents of fenugreek seed include volatile oils, alkaloids, saponins, saponogenins, flavanoids, and mucilage [6]. The seeds are reported to be rich in polyphenolic flavanoids [7]. Looking towards the pharmacological and therapeutic values of this plant, the present study has been carried out to access the radioprotective role of seeds of fenugreek against radiation induced hematological alterations in mice.

MATERIALS AND METHODS

Animals

Animal care and handling were performed according to the guidelines set by the World Health Organization (WHO), and the Indian National Science Academy (INSA). Male Swiss albino mice (*Mus musculus*), 6-8 weeks old and weighing 22–25 g from an inbred colony, were used for the present study. These animals were maintained under controlled conditions of temperature and light (light: dark, 10 hours: 14 hours.). They were provided standard mouse feed (procured from Ashirwad Industries Chandigarh, India) and water *ad libitum*. Tetracycline water once a fortnight was given as preventive measures against infections. The Departmental Animal Ethical Committee approved the present study.

Irradiation

Cobalt teletherapy unit (ATC-C9) at the Cancer Treatment Centre, Radiotherapy Department, SMS Medical College & Hospital, Jaipur, was used for irradiation. Unanaesthetized animals were restrained in well-ventilated perspex boxes and exposed to gamma radiation at the distance of 77.5 cm from the source to deliver the dose-rate of 1.32 Gy per minute.

Preparation of fenugreek seeds extract

The seeds of fenugreek (Methi) plant were collected after proper identification (Voucher No. RUBL20597) by a taxonomist in Herbarium of Botany Department, University of Rajasthan, Jaipur. The plant seeds were powdered in a mixture and the extract was prepared by refluxing with the double distilled water (DDW) for 36 hours (3–12) at 40 °C. The liquid extract was cooled and concentrated by evaporating its liquid contents in vacuum and freeze dried. The prepared fenugreek extract (TFE) was stored at low temperature until further use. Such extract was dissolved in DDW prior for the oral administration in mice.

EXPERIMENTAL DESIGN

Determination of optimum dose of TFE against irradiation

The dose selection of the TFE was carried out on the basis of a drug tolerance study. For this purpose, the various doses of TFE (25, 50, 75, 100, 150, and 200 mg/kg b. wt.* per day) were tested in Swiss albino mice for their effects on the tolerance to 8.0 Gy gamma radiation, and the survival rate (28, 45, 60, 88, 52, and 48 % survival, respectively) of the animals was observed. The most optimum and tolerable dose of TFE (100 mg/kg b. wt.) was selected and used for further detailed experimentation.

Modification of radiation response

Mice selected from an inbred colony were divided into following four groups.

Group I ($n = 5$): Mice of this group were administered orally with DDW, volume equal to TFE (100 mg/ kg b. wt. per animal) and serve as control.

Group II ($n = 5$): These mice were administered TFE by oral gavage once daily at a dose of 100 mg/kg b. wt. per animal for 5 consecutive days to serve as TFE treated group.

Group III ($n = 30$): Mice received DDW volume equal to TFE for 5 consecutive days (as in Group I). Half an hour after the last administration of DDW, animals were exposed to 5 Gy gamma rays.

Group IV ($n = 30$): Mice were treated with TFE orally for 5 consecutive days (as in Group II) and were exposed to gamma radiation half an hour after the last administration of TFE on day 5th.

All these animals were observed daily for any sign of sickness, morbidity, behavioural toxicity, and mortality. A minimum of 4 animals from each groups (III and IV) were necropsied on 12 hours, days 1, 3, 7, 15, and 30 post-treatment intervals to evaluate hematological and biochemical alterations.

Hematological study

Blood was collected from the orbital sinus of animals from each group in a vial containing 0.5 M EDTA (ethylene diamine tetra acetic acid). Total number of erythrocytes (RBC), erythrocytes sedimentation rate (ESR), hematocrit (Hct), and hemoglobin (Hb) percentage were estimated by adopting standard procedures.

Biochemical study

Lipid peroxidation assay: The lipid peroxidation (LPO) level in liver and blood serum was measured in

* mg/kg b. wt. means mg/kg of body weight

terms of thiobarbituric acid reactive substances (TBARS) by the method of Okhawa *et al.* [8] with or without TFE after 24 hours of exposure of animals to 5 Gy gamma radiation. The absorbance was read at 532 nm using a UV-VIS Systronics spectrophotometer (manufactured at Ahemdabad, India).

Glutathione assay: The hepatic level of reduced glutathione (GSH) was determined by the method of Moron *et al.* [9]. The GSH content in blood was measured spectrophotometrically using Ellman's reagent with 5-5, dithiobis-2-nitrobenzoic acid (DTNB) as a colouring reagent, according to the method of Beutler *et al.* [10]. The absorbance was read at 412 nm. GSH in liver and blood was measured after 24 hours of irradiation of mice to 5 Gy gamma rays with or without TFE.

Statistical analysis

The results obtained from the experiment at various necropsy intervals were expressed as mean \pm standard error (S.E.). The statistical difference between various groups were analysed by the Student's 't' test and the significance was observed at different levels as $p < 0.05$, $p < 0.01$, and $p < 0.001$.

RESULTS

All hematological parameters (*i. e.* RBC, ESR, Hb, and Hct percentage) did not show any noticeable change from 24 hours. to day 30th after Sham-irradiation (Group I). TFE treatment to Swiss albino mice (Group II) did not exhibit any significant alterations in these hematological parameters as compared to control irradiated animals.

Animals subjected to 5 Gy gamma rays (Group III) exhibited mild signs and symptoms of radiation sickness. Food or water consumption was reduced and some appeared to be lethargic; however, no mortality was evident in any of the group. No adverse effects in terms of sickness were observed in animals treated with drug alone, and also these did not show significant change in body weight, urination, and defecation pattern.

Following irradiation, a significant decrease in total erythrocyte count was recorded as early as at 12 hours (Group III) and continued up to day 3 post-irradiation. Thereafter, such cells showed a slight recovery from day 7 onwards but a normal value could not be achieved even till the last autopsy interval (*i. e.*, day 30). In the animals of Group IV, a significant ($p < 0.001$) increase in red cell counts with respect to irradiated control was noticed during the entire period of study by restoring almost a normal value on the last autopsy interval, *i. e.*, day 30 (fig. 1).

Similarly, a significant fall in haemoglobin concentration was observed in mice exposed to 5 Gy gamma rays. Later, a slight increase was observed

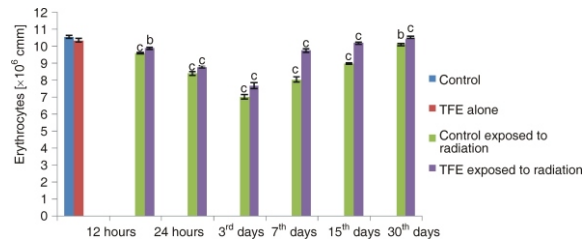


Figure 1. Variations (mean \pm S. E.) in the erythrocytes count [10^6 cmm] in the peripheral blood of mice after exposure to 5.0 Gy gamma radiation with (experimental) or without (control) *Trigonella foenum* extract (TFE). Autopsy interval: a = p 0.05, b = p 0.01, c = p 0.001

from day 7 but the values remained below normal till the last autopsy day. Animals pre-treated with TFE exhibited a higher hemoglobin concentration than Group III, and the values were found to be near normal by the end of the experiment (fig. 2).

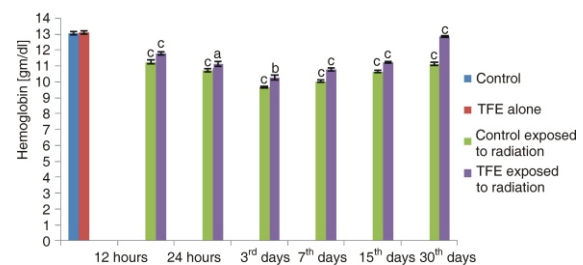


Figure 2. Variations (mean \pm S. E.) in the hemoglobin level [gm/dl] in the peripheral blood of mice after exposure to 5 Gy gamma radiation with (experimental) or without (control) *Trigonella foenum* extract (TFE). Autopsy interval: a = p 0.05, b = p 0.01, c = p 0.001

Further, hematocrit percentage was registered significantly lower ($p < 0.001$) in irradiated control Group III, with maximum decline on day 3. In Group IV, hematocrit values were higher than the control with a recovery from day 7 and reached normal by day 30. On the contrary, TFE pre-treated irradiated animals exhibited significantly higher hematocrit values throughout the experiment (fig. 3).

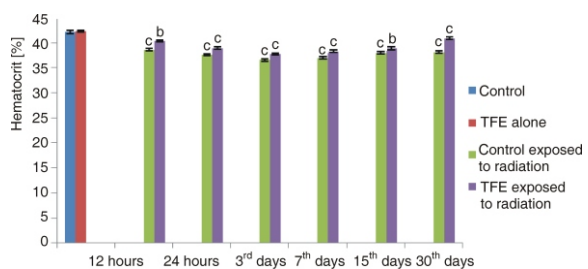


Figure 3. Variations (mean \pm S. E.) in the hematocrit [%] in the peripheral blood of mice after exposure to 5.0 Gy gamma radiation with (experimental) or without (control) *Trigonella foenum* extract (TFE). Autopsy interval: a = p 0.05, b = p 0.01, c = p 0.001

In Group III, the erythrocyte sedimentation rate exhibited a significant rise above normal and reached maximum on day 7. Later, a gradual decline was noticed on day 15, without attaining a normal value even till the last autopsy interval. In TFE pre-treated animals (Group IV), the increase in ESR was significantly lesser than Group III and the value did not rise much higher than the normal even up to day 30 (fig. 4).

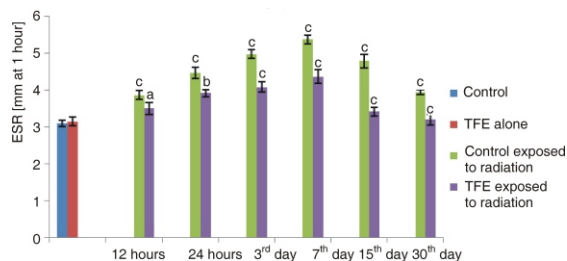


Figure 4. Variations (mean \pm S. E.) in the erythrocyte sedimentation rate (ESR) [mm/hour] in the peripheral blood of mice after exposure to 5.0 Gy gamma radiation with (experimental) or without (control) *Trigonella foenum extract* (TFE). Autopsy interval: a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$

There was no significant difference in the levels of GSH, and LPO in blood/serum content between Sham-irradiated (Group I) and TFE alone treated (Group II) animals; however, TFE pre-treated irradiated animals exhibited a significant elevation ($p < 0.001$) in glutathione (blood and liver) as compared with Group III, but the values remained below normal (figs. 5, 6).

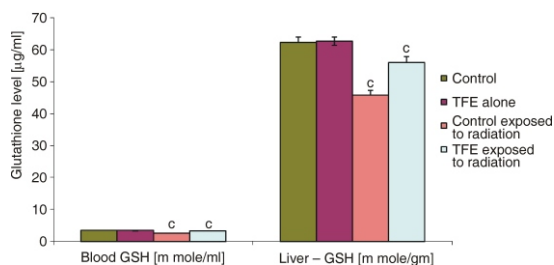


Figure 5. Reduced glutathione (GSH) level in blood and liver of Swiss albino mice after exposure to 5 Gy gamma rays with (experimental) or without (control) *Trigonella foenum extract* (TFE). Autopsy interval: a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$

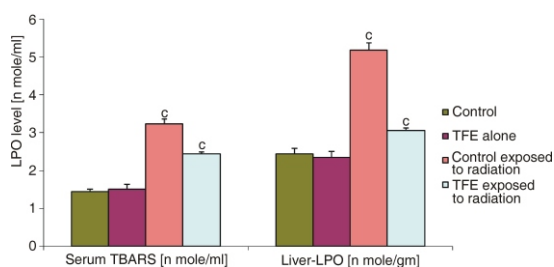


Figure 6. Lipid peroxidation (LPO) level in serum and liver of Swiss albino mice after exposure to 5.0 Gy gamma rays with (experimental) or without (control) *Trigonella foenum extract* (TFE)

A significant increase ($p < 0.001$) in blood and hepatic lipid peroxidation levels was noted in gamma irradiated animals (Group III) as compared to normal. However, these levels declined significantly in the TFE pre-treated irradiated (Group IV) animals.

DISCUSSION

Since the hematopoietic system has a high level of cell turn over, it is among the most radiosensitive tissues in the body. Radiation induced hematological alterations have been extensively studied [11, 12] to understand the intricate pathways involved in radiation mediated damage and to develop novel radioprotective drugs for modulation of radiation induced lethal damage, thereby providing better treatment modalities. In the present study, a significant loss in body weight was evident in control (radiation alone) animals. A dose dependent weight loss in mouse has also been reported [13]. In untreated irradiated animals, body weight decreased drastically and this can be attributed to reduced food and water intake [14], fluid loss by diarrhoea and diminished absorption capacity of the GI tract. Haematopoietic syndrome is characterized by symptoms such as weight loss, irritability, lethargicity, ruffling of hair, emaciation, and epilation [15]. The primary cause of mortality in irradiated control in the present study may be due to sepsis, from opportunistic infections and increased translocation of bacteria across the gastrointestinal mucosa as also suggested by others [16].

In the present investigation, a significant deficit in haematological constituents of peripheral blood (RBC, WBC, Hb, Hct) of irradiated control was recorded as compared to the TFE pre-treated animals. However, a sharp decline in erythrocytes, hematocrit, and hemoglobin was observed up to 3rd day of post-irradiation in both the groups. Exposure to ionizing radiations induces a dose dependent decline in circulating hematopoietic cells, not only through reducing bone marrow cell production but also by redistribution and apoptosis of mature cells [16-18]. Hematocrit is the percentage of whole blood that is made up of cells and decrease in its value below normal indicates anaemia. Another measure of anaemia is a decrease in hemoglobin percentage [19]. In the present study, it has been observed that the hemoglobin level significantly declined following irradiation with 5 Gy. These observations are in accordance with the findings of others [20, 21]. The decrease in Hb content is attributed to decline in number of RBC in peripheral blood. In TFE pre-treated animals, Hb values were higher than the irradiated control group which showed significant protection of RBC by TFE. A considerable decline in Hct was also recorded in the experimental group which is related to failure of erythropoiesis and increased plasma volume.

In the present study, TFE might have offered protection to bone marrow and erythropoietic cells which subsequently maintained the normal values of RBC, ESR, Hb, and Hct in blood. One of the basic mechanisms of radiation damage is generation of highly reactive oxygen species (ROS). The presence of polyunsaturated fatty acids (PUFA) in cell membrane makes it highly susceptible to oxidative attack leading to a chain reaction called as lipid peroxidation [22]. It also disturbs the antioxidant defense system and reduces the intracellular concentration of GSH. However, TFE treatment did not significantly alter the lipid peroxidation level in unirradiated animals but it significantly lowered the radiation induced LPO in experimental group. Inhibition of LPO in bio membranes can be achieved by antioxidants [23, 24]. GSH is a versatile protector and executes its radioprotective function through free radical scavenging, restoration of damaged molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state [25]. The present study demonstrates significant reduction in blood as well as liver GSH following radiation exposure may be due to enhanced utilisation of the antioxidant system to detoxify the free radicals generated by radiation. Oral administration of TFE during radiation exposure protects the endogenous GSH depletion.

Natural antioxidants exhibit a long window of protection, *i. e.*, they provide some protection when administered hours before or after radiation exposure. Exogenous administration of antioxidants, such as glutathione, superoxide dismutase (SOD), antioxidant vitamins (A, C, and E), the disulfide lipoic acid, as well as substances that mimic or induce activity of endogenous antioxidant systems (*e. g.*, selenium, zinc, and copper salts and metal complexes), have shown protection against hematopoietic syndrome death [26-34]. The whole extract of fenugreek has been reported to contain several bioactive components such as glycosides, alkaloids, bitter principle crystalline compounds which elicit protection against several stress and pathological conditions by acting through different mechanisms such as antioxidant defense system [35], stimulation of cell proliferation immunomodulatory and anti-inflammatory activity. The important ingredients present in TFE may be responsible to scavenge radiation induced free radicals, inhibit GSH depletion and combat oxidative stress as well as hematological lesions. The hematopoietic stem cells could be protected against radiation by TFE, which might be responsible for the increased blood constituents. It was also evident in the increased spleen weight and number of radiation-induced spleen colonies (CFU-S) in TFE and radiation combined group as reported in our previous study [36].

CONCLUSIONS

The inherent antioxidant abilities of TFE to bring about a post-irradiation hematopoietic recovery at therapeutic dose make it as a useful herbal drug for achieving radioprotection. From the promising results obtained in the present study, it can be anticipated that herbal preparations from fenugreek could be widely exploited in clinics and for protection of individuals in the situation of radiological emergency.

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УТИЦАЈ ЕКСТРАКТА БИЉКЕ *TRIGONELLA FOENUM GRAECUM* (МЕТХИ) НА ХЕМАТОЛОШКЕ ПАРАМЕТРЕ МИШЕВА ИЗЛОЖЕНИХ ГАМА ЗРАЧЕЊУ

У овом раду приказани су резултати испитивања радиопротективног потенцијала екстракта семена биљке *Trigonella foenum graecum* (ТФЕ) на хематопоезно ткиво. Испитивања су спроведена на мишевима. Испитиваној групи мишева орално је аплицирана оптимална доза ТФЕ 100 mg/kg телесне масе, у континуитету од 5 дана. Контролној групи мишева дата је дестилована вода. Пола сата након апликовања последње дозе, мишеви су озрачени гама зрачењем, дозом 5 Gy. У временским интервалим 12 часова до 30 дана животиње су жртвоване и обављена су хематолошка и биохемијска испитивања. Одређен је укупни број еритроцита, вредност хемоглобина и хематокрит. Утврђено је да су сви наведени хематолшки параметри очувани само у групи животиња које су претретиране екстрактом семена биљке *Trigonella foenum graecum*. У третираној групи животиња динамика опоравка хематолошких параметара је била таква да су базалне вредности постигнуте на крају експеримента. Из добијених резултата може се закључити да ТФЕ протективно делује на хематопоезни систем, и да се може користити у сврху превенције хематолошких дисфункција које настају као последица озрачивања.

Кључне речи: гама зрачење, хематолошки параметри, Trigonella foenum graecum, швајцарски бели миш
