RADIOPROTECTIVE POTENTIAL OF *TRIGONELLA FOENUM GRAECUM* SEEDS EXTRACT

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Summary

The use of plant and natural products may be beneficial in protecting against the radiation induced damage, as they are less toxic or practically non-toxic at their optimum protective doses. Therefore, screening of natural products presents a major avenue for the discovery of new radioprotective drugs. Research has proved that the *Trigonella foenum-graecum* can inhibit the cancer of liver, lower blood cholesterol levels and also have an anti-diabetic effect. In the present study, this plant has been trialed to evaluate its possible radioprotective action. For this purpose, mice were divided into six groups and administered with *Trigonella foenum* extract (TFE) (25, 50, 75, 100, 150 and 200 mg/kg b.wt. / animal/ day) for five consecutive days. Thirty minutes after the last administration, these were exposed whole-body to 8 G_Y gamma radiation. These mice exhibited 28, 45, 60, 88, 52 and 48 per cent survival respectively. Hence, the dose 100 mg/kg b. wt. was found to be the optimum dose. On the basis of survival data DRF was evaluated as 1.66. Furthermore, mice treated with (experimental) or without (control) optimum dose of TFE, were exposed whole-body to 7.5 G_Y gamma radiation. Lipid peroxidation (LPO) level was significantly reduced in experimental animals as compared to irradiated control. On the contrary, GSH level was significantly increased in TFE treated animals than the irradiated control.

Keywords: Trigonella foenum, irradiation, radioprotection, lipid peroxidation, glutathione, spleen Colony assay

Introduction

With increasing use of radiation for the medical diagnostic and treatment purposes, it is essential to protect humans against deleterious effects of radiation. In addition to its utility in cancer treatment, an efficient and non-toxic radioprotectors could also prove useful in occupational settings, where ionizing radiation are used or in accidental exposures which leave radioactivity in the environment. Radioprotectors are chemical compounds that have the ability to reduce the biological effects of ionizing radiation on normal tissues, including lethality, mutagenicity and carcinogenicity [1,2] and have applications in clinical oncology, space travel, radiation site clean-up, radiological terrorism and military scenarios[3]. An ideal radioprotector is relatively non-toxic to normal cells, easy to administer and does not degrade performance nor compromise the therapeutic effects of radiation treatment for cancer patients [4,5]. Several chemical compounds and their analogues have been screened for their radioprotective ability however, their high toxicity at optimum protective doses precluded their clinical use [6,7]. In recent years, it has become well evident that antioxidant phytochemicals are present in plants, fruits and vegetables [2,8,9], therefore, herbal medicine is generally considered a well established form of complimentary medicine.

Fenugreek (*Trigonella foenum-graecum* in latin "Greek hay"), belonging to the family Leguminosae, is one of the oldest medicinal plants of Mediterranean origin and cultivated as a semi-arid crop worldwide. Fenugreek is used both as a herb (the leaves) and as a spice (the seed). The aqueous extracts of seeds and leaves of Fenugreek have been shown to possess hypoglycaemic activity and are nontoxic[10,11,12,13]. Literature survey revealed that fenugreek seeds possess a plethora of benefits under various experimental conditions. The seeds possess significant anti-diabetic, anti-atherosclerotic [14], anti-inflammatory [15], anti-nociceptive [16], anti-ulcerogenic [17] and anti-neoplastic effects [18].

Hence, the wide acceptability, common usage, diverse anti-oxidative and pharmacological properties of Fenugreek aroused an interest to obtain insight into its radioprotective potential.

Materials & Methods

Animals An inbred colony of Swiss albino mice was raised by random breeding of male and female sexes. For this purpose, 5 females and 1 male were kept in polypropylene plastic cages of 15″x 10″x 6″ size having removable wire gauge top. Saw dust was used as bedding material for animals.

The inbred colony was maintained at room temperature of 25 ± 2^0 C and a light: dark exposure period of 14 hrs: 10 hrs cycle daily. Standard mice feed procured from Ashirwad Industries, Chandigarh, India and tap water *ad libitum* were provided to them. Tetracycline water once a fortnight was given as precaution

against infection. Male Swiss albino mice, 6-8 weeks old weighing 23±2 gms, from the above colony were used for the present study.

Irradiation The Cobalt teletherapy unit (ACT- C9) at Cancer Treatment Center, Radiotherapy Department, SMS Medical College & Hospital, Jaipur was used for irradiation. Unanasthetized animals were restrained in well ventilated perspex boxes and exposed whole-body to gamma radiation.

Preparation of Fenugreek seeds extract The seeds of *Trigonella foenum-graecum* (Methi) plant were collected after proper identification (Voucher No.) 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 hrs at 40ºC. The liquid extract was cooled and concentrated by evaporating its liquid contents in vacuo and freeze dried. The prepared *Trigonella foenum-graecum* 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

The following experiments were conducted:

1. Determination of TFE tolerance

Mice for this experiment were divided into different groups of 10 animals each and given TFE orally 25, 50, 75, 100, 150 and 200 mg / kg / b.wt / animal / day for five consecutive days. These animals were observed continuously for any sign of sickness, morbidity, mortality, behavioral toxicity and weight change till 30 days of the last oral treatment.

2. Selection of optimum dose of TFE against irradiation

Mice for this experiment were divided into six groups of 10 animals each and treated with TFE at the dose of 25, 50, 75, 100, 150 and 200 mg / kg / b.wt / animal / day for five consecutive days. Thirty minutes after the last administration of TFE, mice were exposed whole-body to 8 G_Y gamma radiation. The animals were observed till 30 days for any sign of radiation sickness, morbidity, mortality, weight change and behavioral toxicity.

3. Endogenous spleen colony assay (CFU-S)

The endogenous spleen colony assay was done with the slight modification of the method of Till and Mac Culloch [19]. Group of 10 animals was irradiated to 10 G_y with or without TFE. These were sacrificed on 10^{th} day after single total body irradiation (TBI) and their spleens were removed, weighed, and fixed in Bouin's fluid and grossly visible nodules on the surface of the spleens were counted.

4. Dose Reduction Factor (DRF) of TFE

In order to establish the survival-dose-response against radiation in the presence or absence of TFE, two groups of animals were used. The mice of one group were given orally double-distilled water (DDW), equal to volume of TFE and were exposed to different doses (2.5, 5, 7.5 & 10 Gy) of gamma radiation, while the animals of other group were given TFE (100 mg/kg b.wt./day) orally for five consecutive days, and were exposed to similar doses of gamma radiation (as in Group-I) after half an hour of the last administration of TFE.

5. Biochemical Analysis

Lipid peroxidation assay: The lipid peroxidation (LPO) level in liver and blood serum was measured in terms of thiobarbituric acid reactive substances (TBARS) by the method of **Okhawa** *et al* [20] in the presence or absence of TFE after 24 hrs of exposure of animals to 7.5 Gy gamma radiation. The absorbance was read at 532 nm using a UV-VIS Systronics Spectrophotometer.

Glutathione assay: The hepatic level of reduced glutathione (GSH) was determined by the method of **Moron** *et al* [21]. 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* [22]. The absorbance was read at 412 nm. GSH in liver and blood both was measured after 24 hrs of irradiation of mice to 7.5 Gy gamma rays with or without TFE.

Statistical Analysis

Student's t-test was employed to analyze the results of significance between irradiated control and experimental groups. Regression analysis was done to obtain LD 50/30 values and to determine dose reduction factor (DRF).

Results

1. Determination of TFE tolerance

No adverse effects were observed in terms of sickness, body weight changes, mortality and visible abnormality throughout the study in animals treated with different doses (25, 50, 75, 100, 150, 200 mg/ kg b.wt/ day) of TFE for 5 consecutive days. These mice were observed till 30 days post-treatment. However, during the entire experiment, no sickness and mortality were observed in any of the above group which indicates that even the high dose of TFE (i.e. 200 mg/kg b.wt / day) is well tolerable in Swiss albino mice.

2. Selection of optimum dose of TFE against irradiation

The optimum dose of TFE against lethal gamma radiation (i.e. 8 Gy) in Swiss albino mice was selected on the basis of survival experiment, where number of

deaths and surviving animals were recorded up to 30 days of irradiation. Mice treated with TFE at doses of 25, 50, 75, 100, 150, 200 mg/kg b.wt./ day for 5 consecutive days prior to irradiation exhibited 28, 45, 60, 88, 52 and 48 per cent survival respectively. The dose 100 mg/kg b. wt. was found to be the optimum dose on the basis of above data, and the further studies were carried out using this dose of TFE as the optimum dose (Figs-1 & 2).

Figure: 1

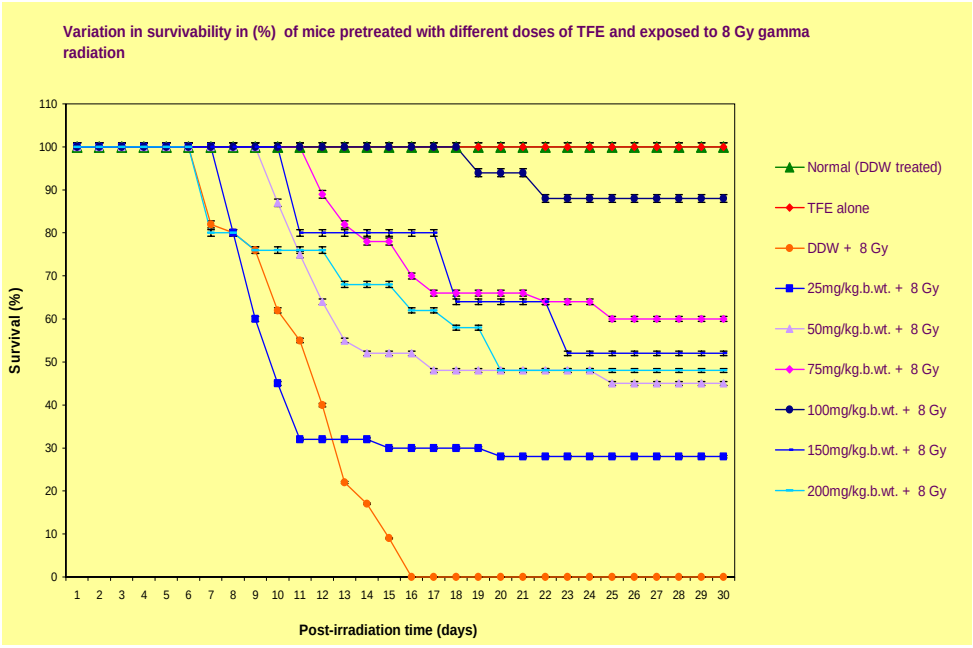
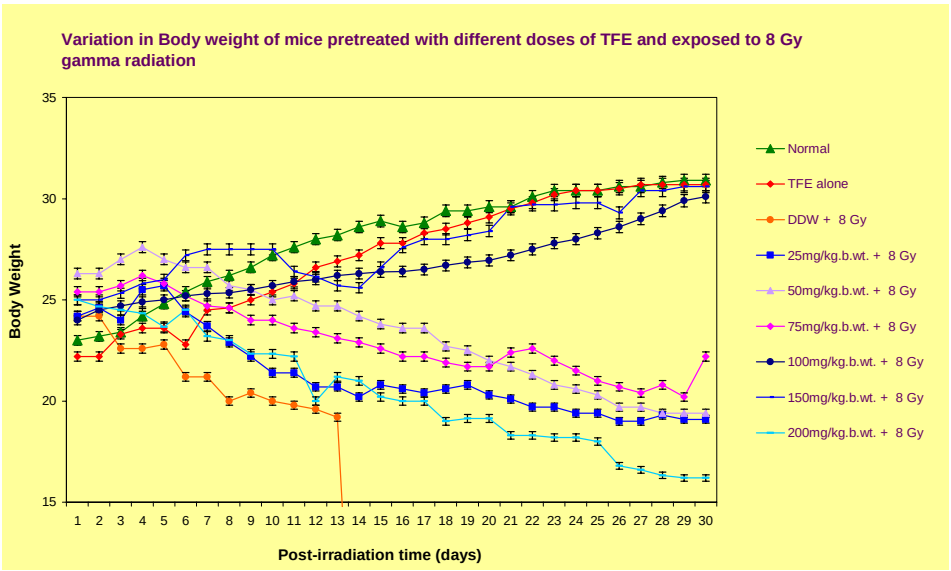


Figure: 2



3. Endogenous spleen colony assay (CFU-S)

Oral administration of TFE to Swiss albino mice for 5 consecutive days before exposure to lethal 10 Gy of gamma radiation was found to be significantly increased in CFU-S as compared to irradiated control. However, no colonies were visible in spleen of the DDW and TFE alone treated animals. Furthermore, a considerable loss in spleen weight following irradiation was noticed at day 10, however, a significant increase in such weight was evident in TFE pretreated irradiated animals. (Table: 1)

Table 1: Variation in Spleen weight and number of colony forming units (CFU) on day 10 in mice after exposure to 10 Gy gamma radiation with (experimental) or without (control)*Trigonella foenum* extract (TFE)

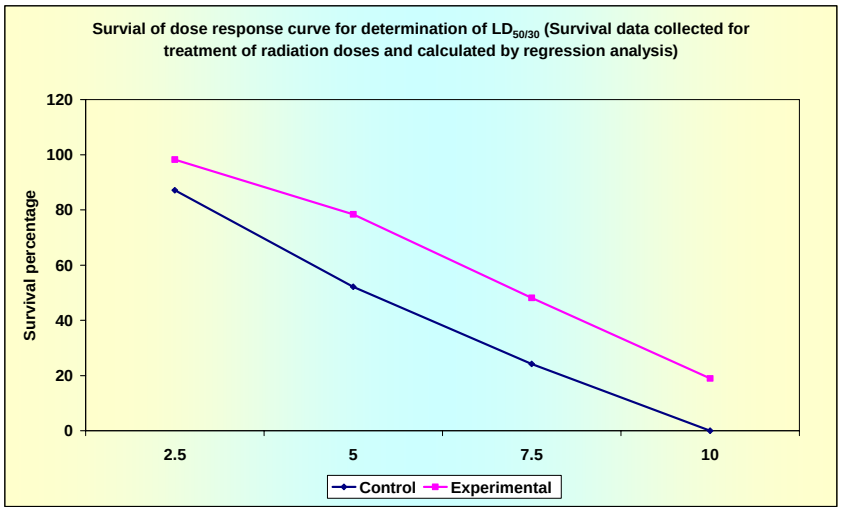
Radiation Dose	Group	Spleen weight (mg)	No. of macroscopic colonies
10 Gy	Control	N.S.	N.S
	Experimental	31.4±0.45	14.4±0.67

Normal Spleen weight: 38.80±0.76
TFE alone treated Spleen weight: 39.2±0.66

4. Dose Reduction Factor (DRF) of TFE

The survival percentage of mice up to 30 days of exposure against each radiation dose was used to construct survival dose-response curves. Regression analysis was done to obtain LD 50/30 values and to determine dose reduction factor (DRF). TFE pretreatment produced a DRF of 1.66 (Fig-3).

Figure: 3

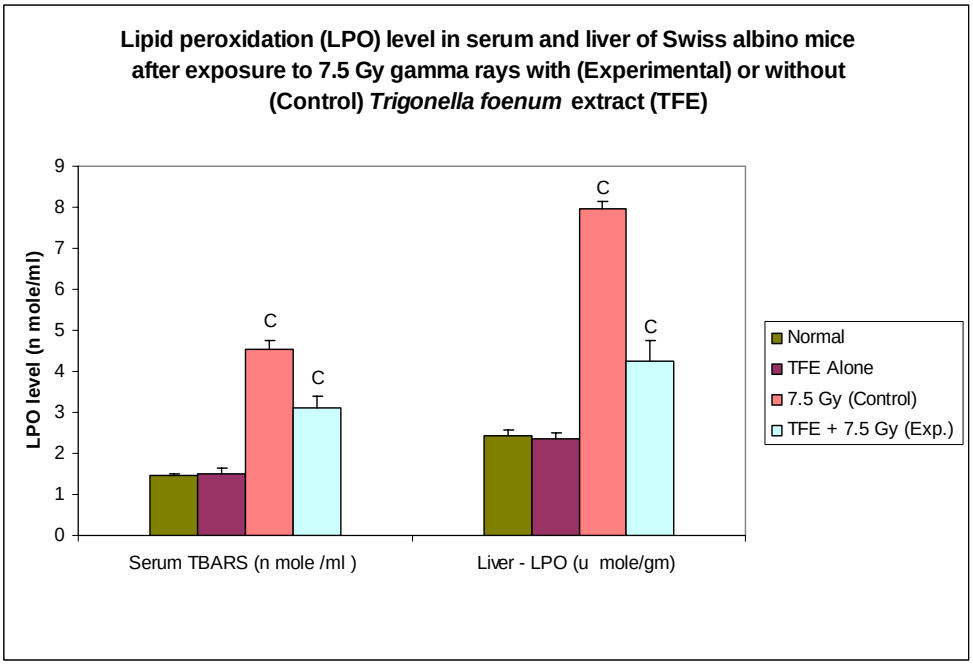


Biochemical analysis

Lipid Peroxidation (LPO)

A considerable rise in LPO level was observed in the blood serum and liver of mice belonging to Group III. On the contrary, administration of *Trigonella foenum* extract (TFE) significantly ($p<0.001$) reduced the level of LPO in Group IV experimental mice as compared to irradiated control (Group-III) (Figure: 4).

Figure: 4

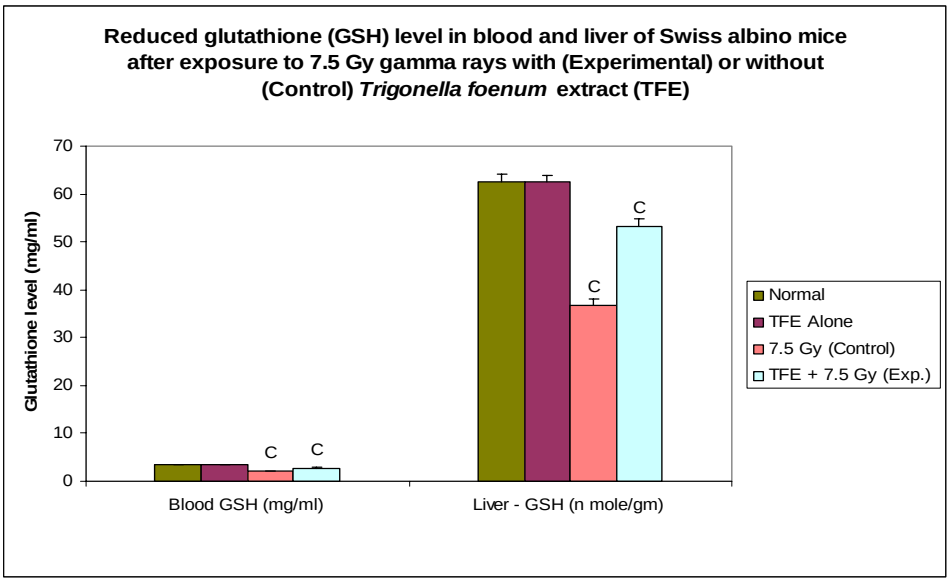


The value represents mean ± S.E. The statistical significance was obtained between Normal V/s Control and Control V/s Experimental **(a = $p<0.05$, b = $p<0.01$, c = $p<0.001$)**

Reduced Glutathione (GSH)

A significant decrease ($p<0.001$) in the level of the non-enzymatic antioxidant protein GSH was noted in Group III (irradiated control) animals than the Groups I and II. On the other hand, GSH level in both the blood and liver of TFE treated animals (Groups IV) was estimated as significantly higher ($p<0.001$) than the irradiated control (Group III) (Figure: 5).

Figure: 5



The value represents mean ± S.E. The statistical significance was obtained between Normal V/s Control and Control V/s Experimental **(a = p<0.05, b = p<0.01, c = p<0.001)**

Discussion

There is a continued interest in and need for the identification and development of non-toxic and effective radioprotective compounds that can reduce the effect of radiation. Such compounds can potentially protect humans against the genetic damage, mutation, alteration in the immune system and teratogenic effects of toxic agents of radiation which act through the generation of free radicals[23]. The prevailing view is that intake of antioxidant nutrients can reduce the risk of free radical-related health problems and may prove to be protective against ionizing radiation.

In the present study, significant radioprotection was achieved when TFE was given orally (100 mg/kg bwt/day) for 5 consecutive days before irradiation. Its radioprotective effect was demonstrated by determination of LD50/30 and evaluation of parameters concerning lipid peroxidation and glutathione levels. A dose dependent mortality and weight loss were recorded on exposure to 8 Gy gamma radiations in 30 days survival assay. Death observed at this radiation dose is mainly attributed to gastrointestinal (GI) [24] and hematopoetic syndrome which resulted in marked loss of water and electrolytes. In untreated irradiated animals, body weight decreased drastically and by day 16th, no animal survived. This can be attributed to reduced food and water intake [25], fluid loss by diarrhea and diminished absorption capacity of the GI tract. TFE pretreated irradiated animals showed recovery in body weight from day 5th onwards and survived till the end of the experiment (i.e. 30 days). The mortality after irradiation could also be due to immunosuppression that increases the chances of infection.

Thus, TFE might have protected irradiated animals against secondary infection and stimulated their fast recovery. This is in agreement with the findings and the use of synthetic chemical compounds like Dilitiazem [26] and plant extracts such as *Mentha piperita* [27], *Emblica officinalis* [28] *Rosemarinus officinalis* [29] as radioprotectors.

Radiation inflicts its adverse effects through the generation of 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 [30]. In our present study, LPO level significantly declined in TFE pretreated animals as compared to irradiated animals. This has been reported as it interferes with the chain reaction by trapping the active oxygen, such as superoxide anion and hydroxyl radicals. Further, the polyphenolic structure of flavonoids partition into the hydrophobic core of the membrane similar to cholesterol and cause a modulation in lipid fluidity [31]. These substances therefore could react with the deeper membrane domain and intracellular structure and protect the cells from oxidant injury. Fenugreek seeds have antioxidant activity and have been shown to produce beneficial effects such as neutralization of free radicals and enhancement of antioxidant apparatus [32,33].

Fenugreek seeds are a rich source of the polysaccharide galactomannan [34]. They are also a source of saponins such as diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens. Other bioactive constituents of fenugreek include mucilage, volatile oils, and alkaloids such as choline and trigonelline [35].

It is well known that free radicals generated during the radiolysis of water play the most significant role in the indirect biological damage induced by ionizing radiation [36]. GSH is one of the major components of cellular antioxidant system. It is the principal non-protein thiol functioning as an antioxidant and as a cofactor for enzymes involved in detoxification of xenobiotics [37]. It plays pivotal role in maintenance of the balance of the cellular redox status, metabolism, transport, catalysis as coenzymes, maintenance of thiol moieties etc. It acts as a radical scavenger due to redox sulphydryl group directly reacting with oxidant and transforms itself into oxidized glutathione. A significant decline in GSH content was noticed in irradiated group as in control. This could be due to its enhanced utilization as an attempt to detoxify the free radicals generated by radiation.

The whole extract of *Trigonella foenum-graecum* has been reported to contain several bioactive components such as glucoside, alkaloids, bitter principle crystalline compounds which elicit protection against several stress and pathological conditions by acting through different mechanisms such as antioxidant defense system [38], stimulation of cell proliferation immunomodulational & anti-inflammatory activity. Reduction in lipid peroxidation and elevation in non-protein sulphydryl groups may also contribute to some extent to its radioprotective activity. Moreover, the hematopoietic stem cells can be protected from radiation-induced free radical damage by TFE,

which was evident in the increased spleen weight and number of radiation-induced spleen colonies (CFU-S) in TFE and radiation combined group.

Conclusion

From the present study, it is evident that *Trigonella foenum graecum* seeds extract has the potential for its use as a radioprotector; however, the further study is going on in this laboratory for its exact mechanism of radioprotection.

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