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Effect of Fenugreek Seeds on Haematogenesis in Irradiated Rats

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Abstract: To evaluate the beneficial effect of fenugreek seeds on rats exposed to irradiation (6.5 Gy) on body weight and hematogenesis at the first and fourteenth day post irradiation. Forty-two healthy adult male Wister rats were divided into four groups: normal control, irradiated control, pre-treated irradiated group and pre and post-treated irradiated group. Body weight gain %, feed efficiency ratio and complete blood count were measured. The results showed that feeding rats on a diet supplemented with fenugreek seeds at a concentration of 5% before and after 14 days of irradiation exposure significantly improved biological evaluation and complete blood count. It can be concluded that the fenugreek seeds significantly induced reduction on body weight and blood content and it can be recommended to use fenugreek seeds before and after exposure to irradiation.

Key words: Fenugreek seeds · Irradiation · Radio- protective · Hematogenesis

INTRODUCTION

The use of ionizing radiation was confirmed to be a two-edged sword since it's revealed by Wilhelm Conrad Roentgen a German scientist in 1895 [1]. Many recent diagnostic and surgical procedures depend on the use of ionizing radiation including nuclear medicine procedures, computed tomography, cardiac catheterization, interventional radiology and electrophysiology procedures [2]. There is particular concern about ionizing radiation since it can cause cellular injury [3]. Damages might occur in living systems by ionizing (removing electrons) from the atoms composing the molecular structures of these systems [4]. The remaining atom positively charged is called a positive ion, or cation, whereas, freed electron is known as a negative ion or anion [5]. Irradiation-induced free radicals affecting the anti-oxidative defense mechanism, causing an increased membrane lipid peroxidation (LPO) and damaged the membrane-bound enzymes [6]. The probable damage depends on the type of radiation and the sensitivity of different body tissues and organs [7]. Recent studies revealed significant association between exposure to radiation and decreased on body weight of irradiated bodies, radiation induced reduction in body final weight which also accompanied with decreasing food intake and accordingly, as well as food efficiency ratio [8, 9, 10].

Irradiated rats with (4Gy) gamma rays showed extramedullary hematopoiesis and a failure in lymphocytes in the white pulp [11]. The decrease in white blood cells differential count is consequence of radiation-induced lipid peroxidation and damage of cell membranes. The reduction in hemoglobin content might be attributed to the decline in number of red blood cells [12]. Lymphocytes have been found to be one of the most radiosensitive mammalian cells [13]. Thiol-dependent enzymes in endogenous cellular such as reduced Glutathione (GSH) play an important role in irradiation response. It has a role in preventing normal tissue by activated antioxidant enzymes [14]. Natural food sources containing thiol groups are essential for the maintenance of cellular structure and function [15]. Among all the antioxidants available in the body, thiols constitute the major portion of the total body antioxidants and they play a significant role in defense against reactive oxygen species. Either total thiols composed of both intracellular and extracellular thiols in the free form as oxidized or reduced glutathione, or thiols bound to proteins [16]. Amongst the biological thiols the high levels of GSH was found in fenugreek extracts (519±52 nM/g dry weight) and showed the highest levels of HCYS, (62 nM/g dry weight) [17]. Fenugreek (*Trigonella-foenum-graecum* L.) is one of the family Fabaceae (Leguminosae) [18], it is a popular medicinal herb extensively used as a food in Indian

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sub-continent and has been used in Indian system of medicine for centuries [19]. Recent researches identified antioxidant, hepatoprotective, anticarcinogenic and other miscellaneous medicinal effects of fenugreek [20]. Fenugreek extracts have been shown to be neutralizing of free radicals and enhancing of antioxidant status [21]. It was also showed radio protective activity [22]. The aqueous extract of fenugreek seeds containing thiols including glutathione GSH (519 nM/g), cysteine CYS (57nM/g), homocysteine HCYS (62 nM/g) and γ-glutamyl cysteine GGC (42 nM/g) [17].

Therefore, the present study was carried out to determine the potential effect of Fenugreek seeds (*Trigonella-foenum-graecum* L.) against radiation on body weight reduction and affecting blood contents in Wister rats.

MATERIALS AND METHODS

All experimental protocols were approved by the Research Ethics Committee (REC) – Faculty of Medicine-King Abdulaziz University, Jeddah, Saudi Arabia. Forty-two healthy adult male Wister rats aged 6-7 weeks, weighing 150-180g were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were housed in a well-ventilated, temperature-controlled room at 23±3°C and the mean relative humidity was 60% (range from 50 to 70) with a 12h light-dark cycle. All rats were freely fed on normal rodent pellets diet and clean water offered *ad-libitum* for one week before starting the experiment for acclimatization. Rats were distributed randomly into three main groups as follows:

Group (1): Normal Control Group (n=12): rats were fed on basal diet for four weeks
Group (2): Irradiated Control Group (n=12): rats were fed on basal diet and exposure to a single sub-lethal dose (6.5 Gy) after 14 days and continue on basal diet for 14 days.
Group (3&4): Treated Irradiated Groups (n=18); rats were fed on basal diet with 5% fenugreek for 14 days and exposure to a single sub-lethal dose (6.5Gy).

After exposure to irradiation, rats were divided into two sub-groups:

- Pre-Treated Irradiated (n=6) rats were fed on basal diet only for 14 days.
- Pre and Post-Treated Irradiated (n=6) rats were fed on basal diet with fenugreek 5% for 14 days.

Fenugreek Preparation and Basal Diet: Whole fenugreek seeds were purified from residuals and washed in cold water, the excess moisture was drain in a strainer and dehydrator at 45°C warm oven. Dried seeds were grounded until fine powder. The fenugreek powder was added to the basal diet at (5%) and all the diet were equaled in nutritional value [23, 24]. The basal diet was provided with standard rat chow pellets obtained from Grain Silos and Flour Mills Organization F-1005, Jeddah, Saudi Arabia, the diet consists of the following ingredients: crude protein 20.0%, crude fat 4.0%, crude fiber 3.5%, vitamin mix 1.0%, mineral mix 3.50%, the remained formula up to 100% corn starch and its energy equals 2850 kcal/kg.

Irradiation: Animals in groups 2, 3 and 4 were placed within acrylic containers and they were exposed to a whole-body X-ray irradiation at a single sub-lethal dose of 6.5 Gy, using a linear accelerator. Animals were returned to their home cages following irradiation. The feed intake (FI), body weight percent (BWG %) and feed efficiency intake ratio (FER) was determined according to the method described by Chapman *et al*. [25] by using the following equations:

$$\text{Body weight gain percent (BWG\%)} = \frac{\text{Final body weight - Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Gian in body weight (g)}}{\text{Feed consumed (g)}}$$

Determination of Complete Blood Count (CBC): Blood was collected from the orbital sinus of rats from each group in a vial containing 0.5 M EDTA. It was determined by using auto hematology analyzer (Mindray BC-2800Vet, China). The methods used to derive CBC parameters are based on the Beckman Coulter method of counting and sizing, in combination with an automatic diluting and mixing device for sample processing and a single beam photometer for hemoglobinometry. Hemoglobin (Hgb), white blood cells count (WBCs) and differential lymphocyte count (Lym) were studied in blood samples.

Histopathological Examination: Histopathological examination through the light microscope was applied on spleen according to the standard procedures of Drury and Wallington [26].

Statistical Analysis: Statistical analyses were processed using Statistical Program of Social Sciences (SPSS) version 22. Results were expressed as mean ± standard error of the mean (SEM). Difference among groups was determined by ANOVA test and statistical was assigned when *P*<0.05.

RESULTS

In the current study, exposing rats to irradiation showed significant (*P*?0.05) reduction in the final body weight accompanied with decreasing DFI and BWG percentage, as well as FER when compared with normal control group. Feeding rats on fenugreek seeds % showed significant (*P*?0.05) improvement in the final body weight, DFI, BWG % and FER in both treated groups compared to irradiated control group (Tables 1 and 2). Regarding the hematological parameters, data in Table 3-5 recorded significant (*P*<0.05) decrease in Hgb, WBC and Lym in irradiated control group compared to normal control group after one and fourteen days post-irradiation. Significant (*P*<0.05) increase in pre-treated irradiated group compared to irradiated control group after one and fourteen days post-irradiation was observed. Microscopically examination of spleen sections of irradiated control rats had shown histopathological changes as necrosis or apoptosis of the T cells (Figs. 1 and 2). Hence, showed a significant decrease in white pulp/red pulp ratio and cellularity of the periarteriolar lymphoid sheath region. In addition to severe hyperemia in the red pulp and sinusoids (or increase in extramedullary hematopoiesis) (Figs. 3 and 4). Pre-treated irradiated group at 1 day post irradiation

Table 1: Effect of exposure to irradiation (6.5 Gy) and feeding fenugreek on initial body weight, final body weight and body weight gain percent (BWG%) in male rats

Parameters --------------------------------- Groups	Initial weight (g)	Final weight (g)	Body weight gain (%)
Normal control	194.10±0.90	317.43±2.12	63.55±1.06
Irradiated control	196.33±2.24	227.10±14.70[a*]	15.71±2.11 [a*]
Pre-treated Irradiated	195.40±1.29	236.00±7.19	20.78±1.40 [b*]
Pre and post-treated irradiated	196.00±0.91	245.87±12.06 [b*]	25.44±0.48 [b*]

Results have been represent Mean ± SEM (mean ± SE) (n=6). (*P* < 0.05)*

[a:]Significantly different from normal control group.

[b:]Significantly different from irradiated control group.

[c:]Significantly different from pre-treated irradiated group.

Table 2: Effect of exposure to irradiation (6.5 Gy) and feeding fenugreek on daily feed intake (DFI) and feed efficiency ratio (FER) in male rats.

Parameters ---------------------------------- Groups	Mean of daily feed intake (g/d)	Feed efficiency ratio (FER)
Normal control	35.30	0.121 ± 0.002
Irradiated control	21.80	0.049 ±0.006[a*]
Pre-treated Irradiated	20.90	0.067 ± 0.005
Pre and post-treated irradiated	21.30	0.081 ±0.002[b*]

Results have been represent Mean ± SEM (mean ± SE) (n=6). (*P* < 0.05)*

[a:] Significantly different from normal control group.

[b:] Significantly different from irradiated control group.

[c:] Significantly different from pre-treated irradiated group.

Table 3: Effect of exposure to irradiation (6.5 Gy) and feeding fenugreek on liver and spleen relative weight in male rats

Parameters ------------------------------------ Groups	Spleen relative weight 1 day post irradiation	Spleen relative weight 14 days post irradiation
Normal control	0.42 ± 0.09	0.43 ± 0.02
Irradiated control	0.36 ± 0.08	0.24 ± 0.01[a*]
Pre-treated Irradiated	0.37 ± 0.01	0.29 ± 0.05
Pre and post-treated irradiated	0.37 ± 0.01	0.34 ± 0.03

Results have been represent Mean ± SEM (mean ± SE) (n=6). (*P* < 0.05)*

[a:] Significantly different from normal control group.

[b:] Significantly different from irradiated control group.

[c:] Significantly different from pre-treated irradiated group.

Table 4: Effect of exposure to irradiation (6.5 Gy) and feeding fenugreek on complete blood count in male rats after 1 day post-irradiation.

Parameters ----------------------------------- Groups	Hgb (12 – 18 g/dL)	WBC (4.1-10.9 K/μL)	Lym (0.6 – 4.1 K/μL)
Normal control	15.27 ±0.12	4.50 ±0.90	1.29 ±0.10
Irradiated control	12.80 ±0.55 ^{a*}	1.48 ±0.14 ^{a*}	0.31 ±0.04 ^{a*}
Pre-treated irradiated	13.93 ±0.71	2.41 ±0.76	1.57 ±0.11 ^{b*}
Pre and post-treated irradiated			

Results have been represent Mean ± SEM (mean ± SE) (n=6). ($P < 0.05$)*

a: Significantly different from normal control group.

b: Significantly different from irradiated control group.

c: Significantly different from pre-treated irradiated group.

Table 5: Effect of exposure to irradiation (6.5 Gy) and feeding fenugreek on complete blood count in male rats after 14 days post-irradiation.

Parameters ----------------------------------- Groups	Hgb (12 – 18 g/dL)	WBC (4.1 - 10.9 K/μL)	Lym (0.6 – 4.1 K/μL)
Normal control	15.27 ±0.22	6.35 ±0.38	2.48 ±0.21
Irradiated control	4.58 ±0.63 ^{a*}	0.75 ±0.15 ^{a*}	0.54 ±0.13 ^{a*}
Pre-treated irradiated	6.24 ±0.23^{b*}	1.00 ±0.04	0.60 ±0.04
Pre and post-treated irradiated	6.86 ±1.15^{b*}	1.65 ±0.16	1.10 ±0.09 ^{b*}

Results have been represent Mean ± SEM (mean ± SE) (n=6). ($P < 0.05$)*

a: Significantly different from normal control group.

b: Significantly different from irradiated control group.

c: Significantly different from pre-treated irradiated group

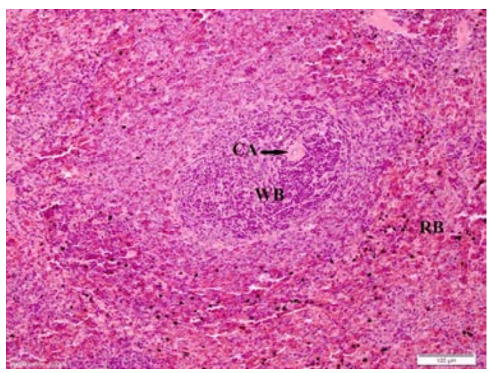

Fig. 1: Light micrograph of rat spleen section from normal control group showing normal spleen architecture: white pulp (WP), central artery (CA) and red pulp (RP). (H & E ×200)

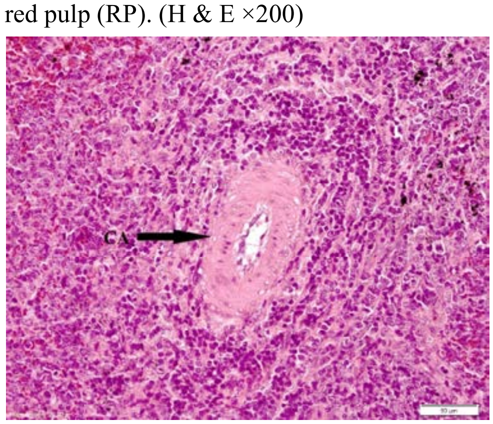

Fig. 2: Light micrograph of rat spleen section from normal control group showing higher magnification of the white pulp showing nodular appearance of lymphoid aggregates around central arteries (CA). (H & E ×400)

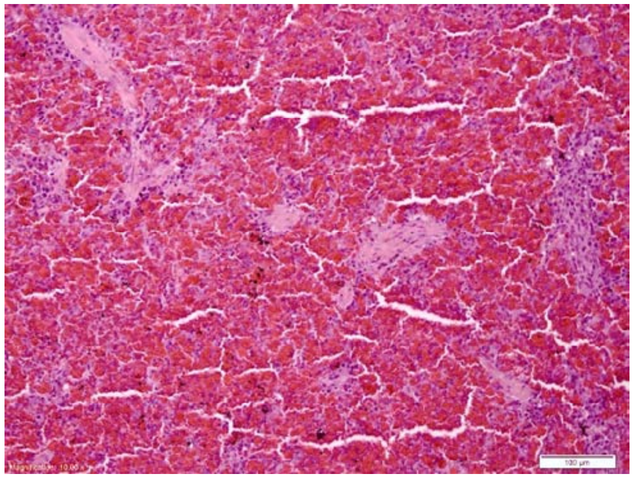

Fig. 3: Light micrograph of rat spleen section from irradiated control group at 1 day after irradiation showing an increase in extramedullary hematopoiesis and a decrease in the lymphocytes in the white pulp (H & E ×200)

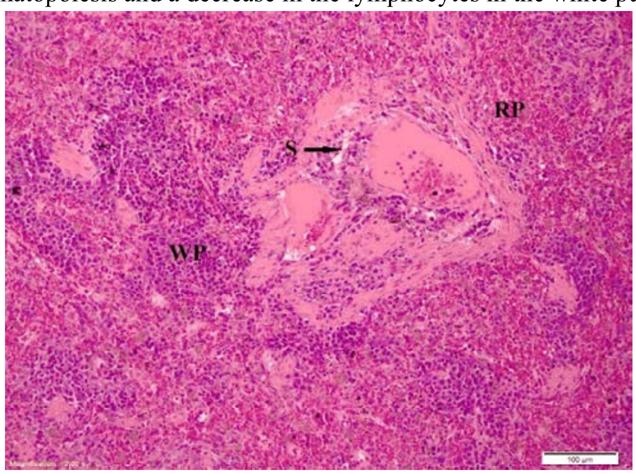

Fig. 4: Light micrograph of rat spleen section from irradiated control group at 14 days after irradiation showing slight improvement: sever hyperemia (or congestion) in the red pulps (RP) and sinusoids (S) accompanied by distorted lymphoid nodules of the white pulp (WP) (H & E ×200)

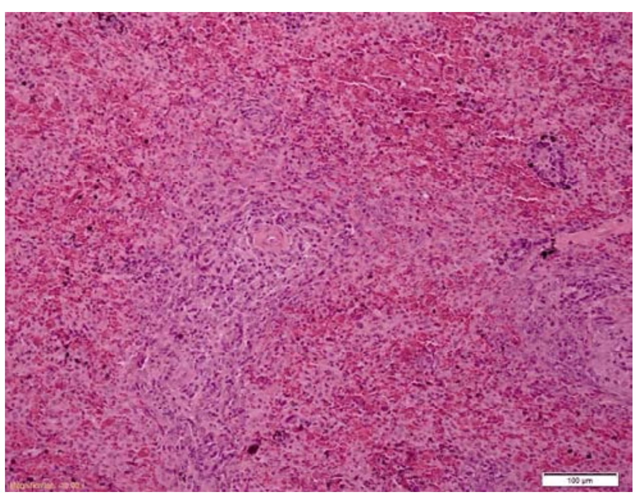

Fig. 5: Light micrograph of rat spleen section from pre-treated irradiated group at 1 day after irradiation showing moderate changes (H & E ×200)

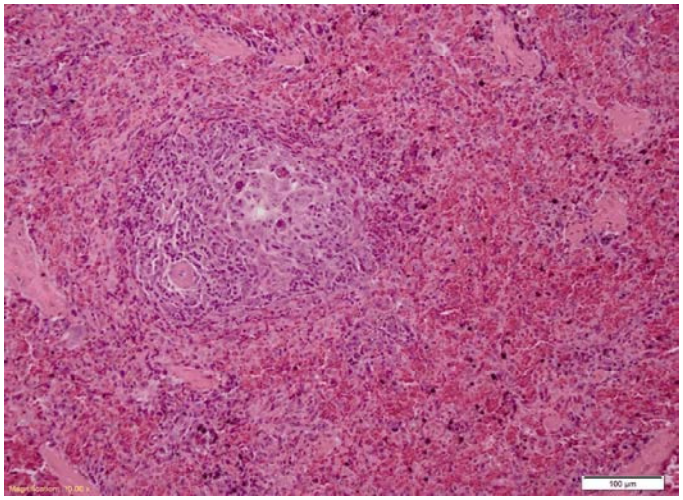

Fig. 6: Light micrograph of rat spleen section from pre-treated irradiated group at 14 days after irradiation showing partial improvement (H & E ×200)

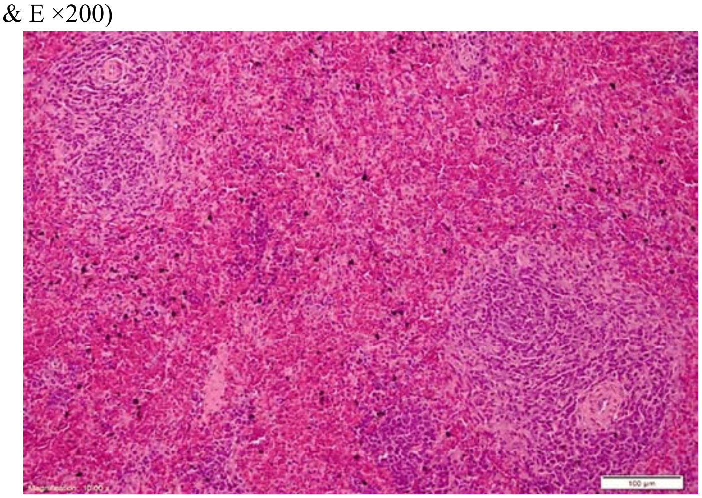

Fig. 7: Light micrograph of rat spleen section from pre and post-treated irradiated group at 14 days after irradiation showing a substantial improvement in the spleen tissue and recovery of its natural constitution (H & E ×200)

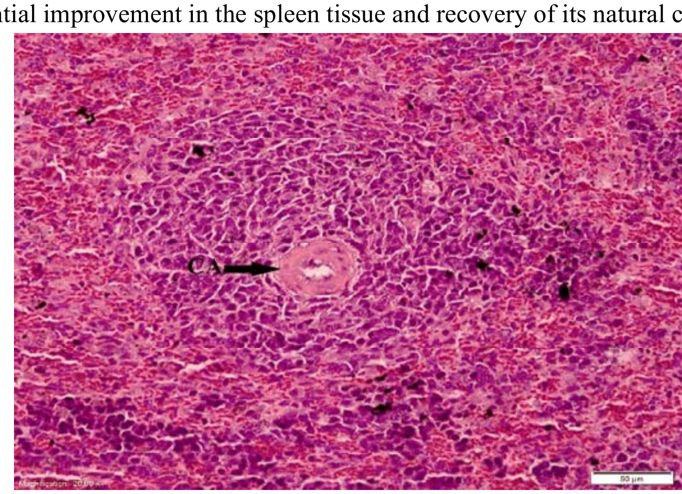

Fig. 8: Light micrograph of rat spleen section from pre and post-treated irradiated group at 14 days after irradiation showing higher magnification recovery of its natural constitution: the nodules of lymphoid in the white pulp aggregates around central arteries (CA) (H & E ×400)

(Figs. 5 and 6) showing moderate changes marked by moderate necrosis of the T cells, as well as slight hyperemia in the red pulps and sinusoids with atrophy of the marginal zone (B-cell area). Partially improvement of structural changes was observed after 14 days post irradiationþ (Fig. 4) but edema and necrosis were still evident. In pre and post-treated irradiated group at 14 days post irradiation, the sections of the spleen had shown a substantial improvement in the spleen tissue and recovery of its natural constitution (Figs. 7 and 8).

DISCUSSION

Exposure of rats to irradiation at 6.5 Gy is known to cause sickness within 3-5 days after exposure. The symptoms included reduction in food and water intake, irritability, weight loss, lethargy, diarrhea and ruffling of hairs [27-30]. The results of the present study showed significant reduction in the final body weight of irradiated control group. The obtained results are in agreement with those obtained by Gupta *et al.* [9], Nwozo *et al.* [10] and Abou-Seif *et al.* [31], who reported a significant decline in body weight of irradiated rats group compared with control non-irradiated rats group. Miyake *et al.* [32] reported that irradiated rats to X-rays 9.0 Gy induced significant and rapid reduction in the body weight until the 4th day of the exposure, then weight decreased gradually until the death of rats. Weight reduction might be occurred through biphasic changes. In the first phase, weight loss might due to the gastrointestinal damages following irradiation. In the second phase, weight loss was associated with decreasing water intake by the rats [33, 34].

Radiation can induce gene expression and secretion of some cytokines, such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α, which involved in the acute inflammatory response to radiation injuries [35]. These cytokines also play a role in the suppression of appetite and cause some degree of weight loss through various pathways. Ghrelin can antagonize the effect of these cytokines on appetite and body weight. Therefore, it might assume that amount of ghrelin production after gastric irradiation is not enough to compensate these inflammatory conditions sufficiently. Whereas, radiation can decrease the concentration of ghrelin and its mRNA expression in the gastric tissue. According to the changes in gastric ghrelin level, body weight and food intake were also reduced in the irradiated group compared to the non-irradiated control group [36]. The diet containing powdered fenugreek seeds 5% showed significant improves in body weight when compared to irradiated group. This result is in agreement with those obtained by Eidia *et al.* [37] and Zargar [38], who reported co-administration of fenugreek with various factors-induced loss weights. However, there is limited literature about fenugreek seeds protection effect against radiation [22, 39, 40]. In previous studies, the optimum dose of fenugreek seeds extract as radioprotection was (100 mg/kg b wt/day) and it was determined based on survival data. There was evidence showed that fenugreek seeds play a strong protective effect against irradiation-induced oxidative stress in adult rats [22]. Hematopoietic tissues and the immune system are highly sensitive to radiation. Radiation damages the hematopoietic system through affecting the hematopoietic stem cells and reduces their cell proliferation capacity. Inhibition of hematopoiesis is one of the clinical symptoms of radiation injury. Acute radiation injury induced by low-dose radiation is usually associated with immune system disease and a decline in white and red blood cells [41]. Exposure to ionizing radiation 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 [42].

In the present study, a significant reduction in hematological constituents was observed in Hb, WBC and Lys of irradiated control group. The results are in agreement with those obtained by El-Masry *et al.* [43], Qi *et al.* [44], Goel *et al.* [45], Verma *et al.* [46] and Ezz [47]. This decrease might be attributed to the high radio sensitivity of haematopoietic tissue [48] and a reduction in the viability of spleen hematopoietic stem cells [49]. Nevertheless, the cellular elements of the blood are particularly sensitive to oxidative stress because their plasma membranes contain a high percentage of polyunsaturated fatty acids [48]. Therefore, the reduction in white blood cells differential count recorded in the irradiated rats might be the consequence of radiation-induced lipid peroxidation and damage of their cell membranes. The reduction in hemoglobin content might be attributed to the decline in the number of red blood cells in peripheral blood [40]. In addition, the reduction in hematocrite might be a consequence of erythropoiesis failure, destruction of mature cells, or increased plasma volume. Meanwhile, the depletion of peripheral blood elements might be a bone marrow syndrome [50].

In the present study, hemoglobin and lymphocytes levels of irradiated rats treated with fenugreek seeds were significantly higher than irradiated control group, showing a significant protection effect of fenugreek seeds on RBC. It was also demonstrated the role of fenugreek seeds in protecting the spleen and increased lymphocyte, suggesting that fenugreek seeds might improve immunity. These observations are in agreement with those obtained by Sindhu *et al.* [51] and Kandhare *et al.* [52], who reported that fenugreek seeds influenced the hemoglobin and lymphocytes count, improving hematopoietic function and survival. Furthermore, a considerable significant lose in spleen following irradiation was noticed at the day 14 post irradiation, which might be explained by the radio sensitivity of the spleen. Radiation therapy can be used to reduce the size of the spleen in patients with leukemia or hypersplenism [53, 54], after a small to moderate dose (4 to 8 Gy) of radiation, the lymphoid tissues are destroyed within an hour and the destructioncontinues for several hours [55, 56, 57]. Rapid phagocytosis occurs and results in complete disappearance of the debris in the spleen within a day after radiation. Regeneration of lymphocytes begins before the end of the first week and continues for one or two weeks [55, 57]. The results are in agreement with those reported by Qi *et al.*[44], Verma *et al.* [46], Rao *et al.* [58], Kunwar *et al.* [59], Zhao *et al.*[60] and Duan *et al.* [61]. On contrary, Fenugreek seeds succeeded to avoid spleen-losing weight when rats fed pre and post-irradiation. The results are in agreement with Chaudhary *et al.* [22], who reported an increase in spleen weight and number of radiation induced spleen colonies in fenugreek seeds extract and radiation combined group. The integrity of the immune system depends on the normal functioning of lymphoid organs so that alterations in the homeostasis of spleen tissues will affect immune responses. The spleen plays an important role in immune functions by proliferating lymphocytes [62]. In the current study, the decrease in lymphocytes might due to oxidative damage in spleen tissues. Furthermore, studies have demonstrated that lymphocytes are considered to be the most sensitive type of blood cells [63] and the earliest blood change following whole body irradiation is lymphopenia [64].

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