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Effect of Fenugreek Seeds Against Irradiation-Induced Oxidative Stress in Adult Rats

[1]Amani A. Alrasheedi, [2]Sarah M. Al-Aamri, [3]Mamdouh M. Eassawy and [4]Fayza A. Bawazeer

[1]Food and Nutrition Department, Faculty of Home Economics,
King Abdulaziz University, Jeddah Saudi Arabia
[2]Nutrition Department, Science and Humanities College, King Khalid University, Abha, Saudi Arabia
[3]Regional Center for Food and Feed (RCFF), Agricultural Research Center, Giza, Egypt
[4]Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract: The present study aims to evaluate the beneficial effect of fenugreek seeds against Irradiation-induced oxidative stress in adult rats. 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. Results showed significant increase (P<0.05) in liver relative weight in irradiated control group after one and fourteen days post-irradiation compared to normal control group. Feeding rats with fenugreek 5% showed significant (P<0.05) decrease in the liver relative weight of the two groups (Pre-treated irradiated and pre and post-treated irradiated) compared to irradiated control group. Exposing rats to irradiation showed significant (P<0.05) increase in the level of liver enzymes (AST, ALT and ALP) in irradiated control group after fourteen days post irradiation. Regarding the changes occurred in antioxidants enzymes in liver tissues, a significant (P<0.05) decrease (GSH & SOD) and increase in (LOP) in irradiated control group. There were significant (P<0.05) increase in GSH & SOD levels of Pre and Post-Treated Irradiated group at both one and fourteen days post irradiation. In irradiated rats, exposure to radiation caused severe liver damage including hepatocyte edema, necrosis of the hepatocytes, karyolysis, proliferation of kupffer cells and dilated sinusoids.

Key words: Fenugreek seeds · Irradiation · Liver enzymes · Oxidative stress

INTRODUCTION

Ionizing radiation produces damages in living systems primarily by ionizing (removing electrons from) the atoms composing the molecular structures of these systems [1]. When ionizing radiation interacts with a cell, ionizations and excitations they are produced in either biologic macromolecules (such as DNA) or water (H_2O), the medium in which the cellular organelles are suspended [1]. The potential damage from an absorbed dose depends on the type of radiation and the sensitivity of different tissues and organs. Based on the site of the interaction, the action of radiation on the cell is classified as either direct (when ionizing particles interact directly with biologic macromolecules such as DNA, RNA, proteins, or enzymes, damage occurs as a result of what is called direct action). On the other hand, indirect (when a specific molecule such as DNA is acted upon by free radicals previously produced by the interaction of radiation with water molecules) [2]. Liver is the most important metabolic organ in the human body. Hepatic injury is associated with distortion of these metabolic functions. Hepatic disease can be evaluated and diagnosed by determining serum concentrations of a number of serum analytes [3, 4]. Ionizing radiation (4.5Gy) is known to induce disturbances in serum liver aspartate aminotransferase and alanine aminotransferase (AST and ALT) and alkaline phosphatase (ALP) activities [5]. Whereas, damaged hepatocytes release their contents including ALT and AST into the extracellular space. The released enzymes ultimately enter into circulation and thereby increase the serum levels of ALT and AST compared to control subjects [6]. Radiation-induced reactive oxygen species (ROS) and reactive nitrogen species (RNS). Nitric oxide and superoxide radicals react to produce reactive peroxynitrite [7, 8], which is known to induced cytotoxicity by interacting with biomolecules like protein, lipids and nucleic acids and induced peroxidation of the

Corresponding Author: Amani A. Alrasheedi, Food and Nutrition Department,
Faculty of Home Economics, King Abdulaziz University, Jeddah, Saudi Arabia.

lipids, DNA and protein thereby producing damaging effects to the cells. Stimulates ROS production damage in mitocondria, increase free radical production and decrease antioxidant production [9, 10] by reducing the intracellular concentration of GSH as well as SOD, GST and CAT activity [11]. One of the most potent exogenously applied against radio protectors are the amino thiols including cysteamine and cysteine. Protection by these molecules has been attributed mainly to the free sulfhydryl group involvement in radical scavenging and hydrogen donation. The role of the amino group within these molecules receives less attention [12]. Nevertheless, 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. Total thiols composed of both intracellular and extracellular thiols either in the free form as oxidized or reduced glutathione, or thiols bound to proteins [13]. Fenugreek (*Trigonella- foenum-graecum* Leguminosse) belongs to the Papilionaceae section of the family Leguminosae [14]. It is one of the most promising medicinal herbs, having nutritional value too [15]. Antioxidant benefits of fenugreek associated with exist phenols and saponins, therefore it used for liver therapy [16]. Daily consumption of fenugreek seeds might reduce the levels of lipid peroxidation products and protein carbonyl content. It promoted mode of action of antioxidant enzymes and restored content of thiol groups [15]. Therefore, the present study aims to evaluate the beneficial effect of fenugreek seeds against Irradiation-induced oxidative stress in adult rats.

MATERIALS AND METHODS

All experimental protocols were approved by the Research Ethics Committee (REC) – Faculty of Medicine - King Abdul Aziz University, Saudi Arabia. Forty-two healthy adult male Wister rats aged 6-7 weeks, weighing 150-180 grams were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdul Aziz 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 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 distributed randomly into three 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 (6.5Gy) 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: The whole fenugreek seeds were purified from residuals and washed in cold water, the excess moisture was drain in a strainer and dehydreter 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 [17, 18]. 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 6.5Gy using a linear accelerator. Animals were returned to their home cages following irradiation.

Determination of AST and ALT Enzyme Activity: according to Reitman and Frankel [19].

Determination of Serum Alkaline Phosphatase Activity: according to Belfield and Goldberg, [20].

Determination of Serum Total Thiols (SH Groups): according to by Sedlak and Lindsay [21].

Determination of Reduced Glutathione (GSH): According to Beutler *et al.* [22].

Assay of Superoxide Dismutases (SODs): According to Nishikimi *et al.* [23].

Determination of Lipid Peroxidation: According to Ohkawa *et al.* [24].

Histopathological Examination: Specimens from each liver and were examined according to Drury and Wallington [25].

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

Results of the current study showed significant increase ($P<0.05$) in liver relative weight in irradiated control group after one and fourteen days post-irradiation compared to normal control group. Feeding rats with fenugreek 5% showed significant ($P<0.05$) decrease in the liver relative weight of the two groups (Pre-treated irradiated and pre and post-treated irradiated) compared to irradiated control group (Table 1). Exposing rats to irradiation showed significant ($P<0.05$) increase in the level of liver enzymes (AST, ALT and ALP) in irradiated control group after fourteen days post irradiation. While, feeding rats on fenugreek 5% significantly decreased the levels of liver enzymes in both treated groups (Table 2). Feeding fenugreek for fourteen days pre and post-irradiation induced a significant ($P<0.05$) increase in total thiols serum level after one and fourteen days post irradiation compared to irradiate control group (Table 3). Regarding the changes occurred in antioxidants enzymes in liver tissues, data in Table 4 shows significant ($P<0.05$) decrease (GSH & SOD) and increase in (LOP) in irradiate control group because of exposing rats to irradiation. There were significant ($P<0.05$) increase in GSH & SOD levels of Pre and Post-Treated Irradiated group at both one and fourteen days post irradiation. A significant ($P<0.05$) increase in (LOP) was observed in pre and post-treated irradiated group after one and fourteen days post irradiation. Histologically, the liver appeared with normal structure in control group (Figs. 1, 2 and 3). In irradiated rats, exposure to radiation caused severe liver damage including hepatocyte edema, necrosis of the hepatocytes, karyolysis, proliferation of kupffer cells and dilated sinusoids (Figs. 4-7).

Table 1: Effect of exposure to irradiation (6.5Gy) and feeding fenugreek on Liver relative weight in male rats

Parameter	Liver relative weight	
Groups	At 1 day post-irradiation	At 14 days post-irradiation
Normal Control	4.68±0.09	4.59±0.09
Irradiated Control	5.05±0.03^{a*}	4.16±0.10^{a**}
Pre-Treated Irradiated		4.49±0.15^{b*}
Pre and Post-Treated Irradiated	4.90±0.03	4.57±0.18^{b**}

Results have been representing Mean ± SEM (mean ± standard error of mean) of 6 animals per group.

ANOVA followed by LSD ($P < 0.05$).

[a]: Significantly different from normal control group.

[b]: Significantly different from irradiated control group.

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

(*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).

Table 2: Effect of exposure to irradiation (6.5Gy) and feeding fenugreek on serum aspartate aminotransferase (AST, ALT and ALP) level in male rats

Parameter	AST (U/L)		AST (U/L)		ALP (U/L)	
Groups	At 1 day post-irradiation	At 14 days post-irradiation	At 1 day post-irradiation	At 14 days post-irradiation	At 1 day post-irradiation	At 14 days post-irradiation
Normal Control	149.50±5.66	132.00±1.79	86.00±0.90	85.33±1.48	240.50±4.57	239.67±8.83
Irradiated Control	161.50±4.57	158.50±6.03^{a***}	96.50±3.80	105.33±9.26^{a*}	261.17±7.23^{a*}	256.50±7.82
Pre-Treated Irradiated		140.33±3.87^{b**}		69.33 ± 5.68^{b***}		180.00±4.83^{b***}
Pre and Post-Treated Irradiated	125.00±3.63$^{b***, c***}$	128.67±4.61^{b***}	67.00±1.83^{b***}	58.50 ± 5.14^{b***}	258.00±9.24	171.67±5.17^{b***}

Results have been representing Mean ± SEM (mean ± standard error of mean) of 6 animals per group.

ANOVA followed by LSD ($P < 0.05$).

[a]: Significantly different from normal control group.

[b]: Significantly different from irradiated control group.

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

(*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).

Table 3: Effect of exposure to irradiation (6.5Gy) and feeding fenugreek on serum total thiols level in male rats

Parameter	Total thiols (µmol/L)	
Groups	At 1 day post-irradiation	At 14 days post-irradiation
Normal Control	58.87±1.62	58.93±1.79
Irradiated Control	30.03±1.03^{a***}	33.72±1.78^{a***}
Pre-Treated Irradiated		46.27±1.85^{b***}
Pre and Post-Treated Irradiated	55.23±1.65$^{b***, c***}$	60.00±0.71$^{b***, c***}$

Results have been representing Mean ± SEM (mean ± standard error of mean) of 6 animals per group.

ANOVA followed by LSD ($P < 0.05$).

[a] Significantly different from normal control group.

[b]: Significantly different from irradiated control group.

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

(*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).

Table 4: Effect of exposure to irradiation (6.5Gy) and feeding fenugreek on liver glutathione (GSH) level in male rats

Parameter	GSH (nmol/g tissue)		SOD (U/mg tissue)		LPO (nmol/g tissue)	
Groups	At 1 day post-irradiation	At 14 days post-irradiation	At 1 day post-irradiation	At 14 days post-irradiation	At 1 day post-irradiation	At 14 days post-irradiation
Normal Control	129.57±1.07	130.33±1.45	3.07±0.08	3.17±0.13	7.13 ± 0.06	7.23±0.06
Irradiated Control	66.30±1.33 ^{a***}	65.95±2.78^{a***}	1.77±0.08 ^{a***}	1.63±0.12^{a***}	34.90±0.90^{a***}	35.33±1.64^{a***}
Pre-Treated Irradiated		76.65±3.94 ^{b**}		1.75±0.08		28.93±3.03^{b**}
Pre and Post-Treated Irradiated	103.63±2.17$^{b***, c***}$	102.03±3.73$^{b***, c***}$	2.67 ± 0.09 ^{b***}	2.58±0.17$^{b***, c***}$	19.30±0.99$^{b***, c***}$	15.53±1.54$^{b***, c***}$

Results have been representing Mean ± SEM (mean ± standard error of mean) of 6 animals per group.

ANOVA followed by LSD ($P < 0.05$).

[a]: Significantly different from normal control group.

[b]: Significantly different from irradiated control group.

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

(*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).

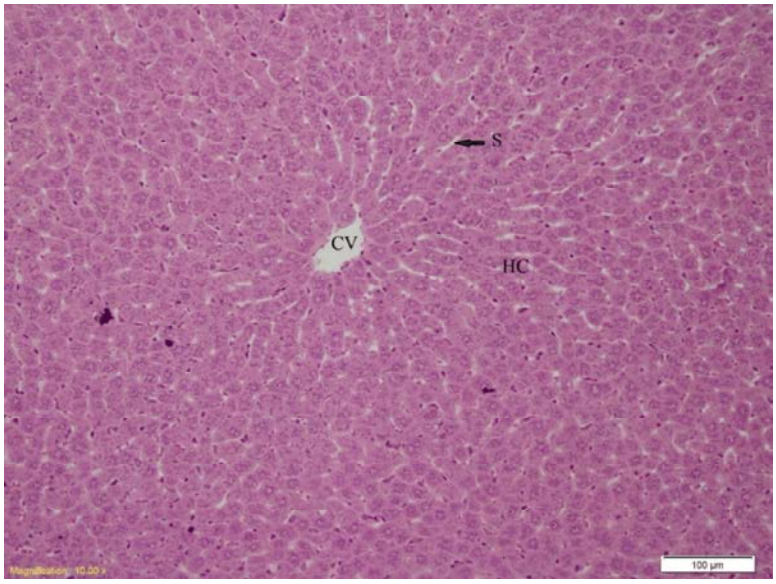

Fig. 1: Light micrograph of rat liver section from normal control group showing normal hepatic architecture: central vein (CV) surrounded by normal hepatocytes in the form of hepatic cords (HC) and hepatic sinusoids (S). (H & E ×200)

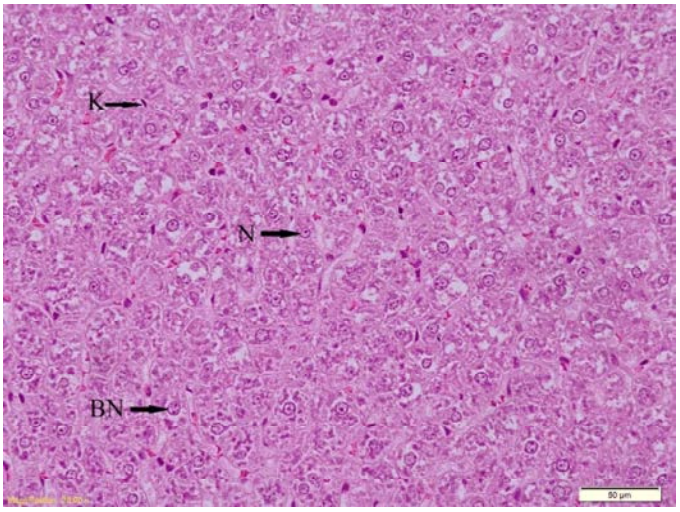

Fig. 2: Light micrograph of rat liver section from normal control group showing higher magnification of the normal hepatic architecture: hepatocyte with their normal nuclei (N), binucleated (BN) and Kupffer cell (K). (H & E ×400)

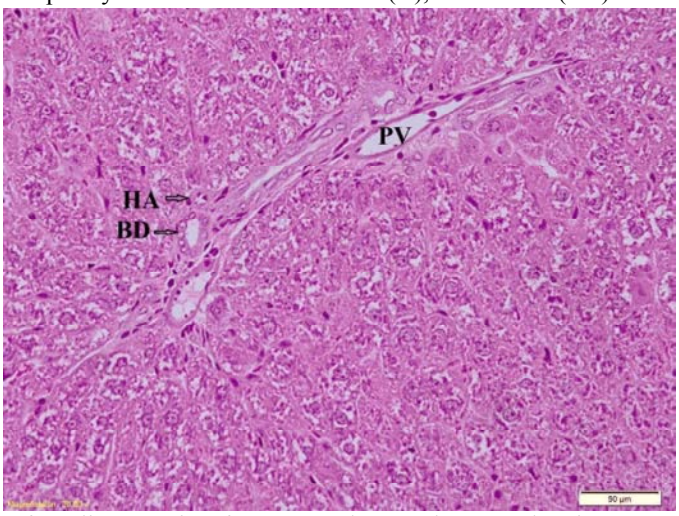

Fig. 3: Light micrograph of rat liver section from normal control group showing normal portal area structures: bile ductless (BD), hepatic artery (HA) and portal vein (PV). (H & E ×400)

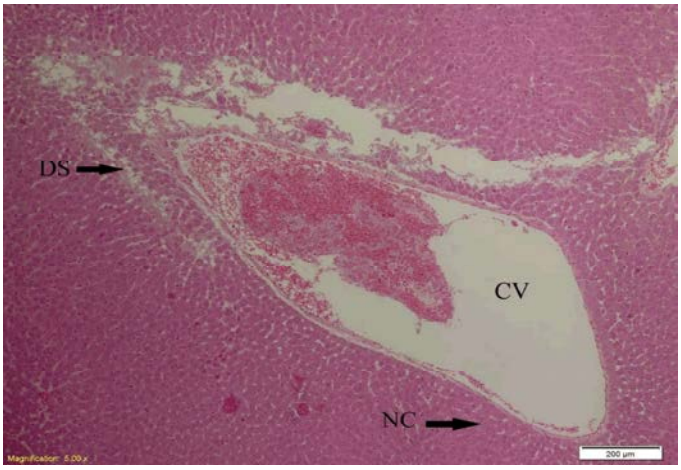

Fig. 4: Light micrograph of rat liver section from irradiated control group at 1 day after irradiation showing morphological changes: congested central vein (CV), dilated sinusoid (DS) and necrotic cells (NC). (H & E ×100)

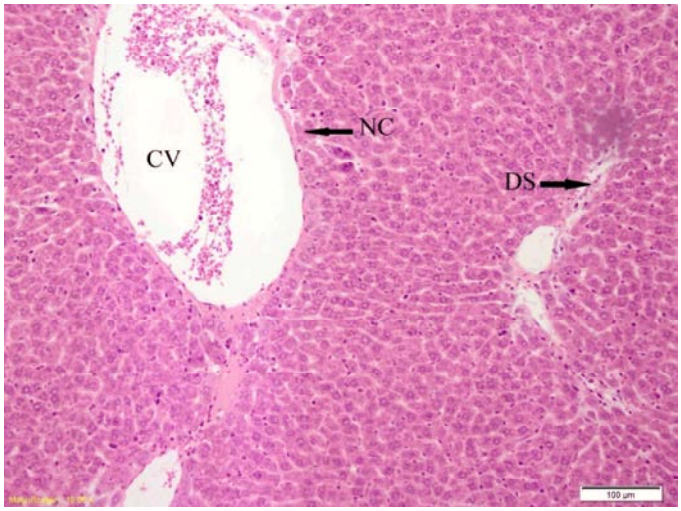

Fig. 5: Light micrograph of rat liver section from irradiated control group at 14 days after irradiation showing morphological changes: congested central vein (CV), dilated sinusoid (DS) and necrotic cells (NC). (H & E ×200)

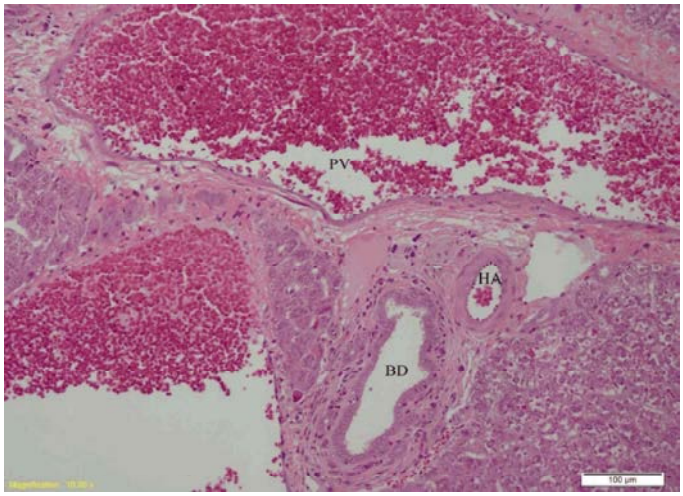

Fig. 6: Light micrograph of rat liver section from irradiated control group at 1 day after irradiation showing morphological changes in the portal area. (H&E × 200)

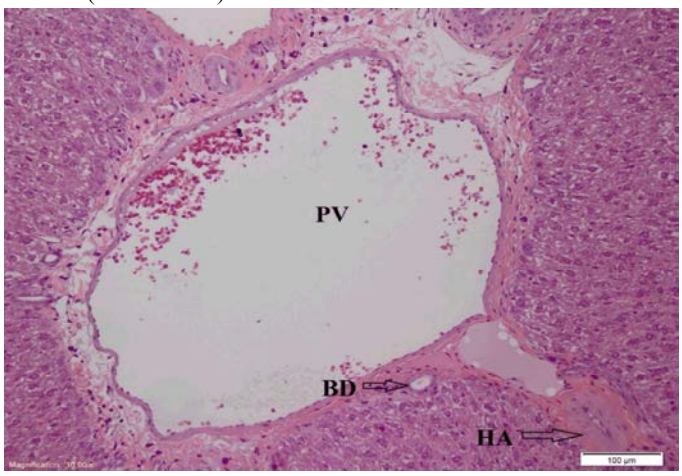

Fig. 7: Light micrograph of rat liver section from irradiated control group at 14 days after irradiation showing morphological changes in the portal area. (H&E ×200)

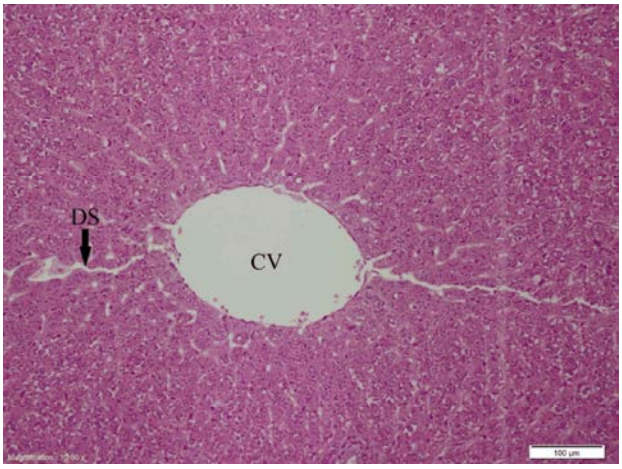

Fig. 8: Light micrograph of rat liver section from pre-treated irradiated group at 1 day after irradiation showing moderate changes: necrotic cells around the central vein (CV) and dilated sinusoid (DS). (H & E ×200)

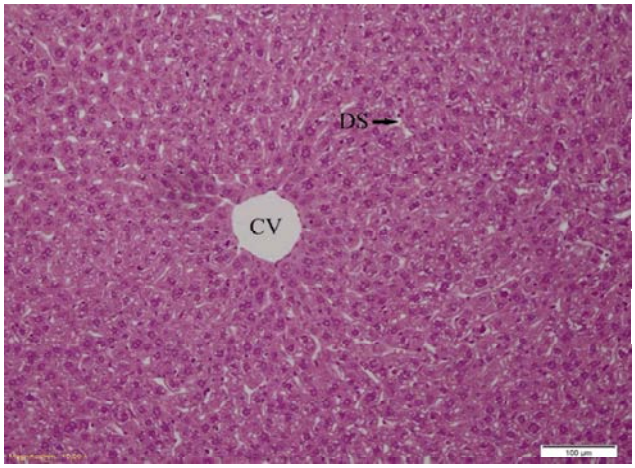

Fig. 9: Light micrograph of rat liver section from pre-treated irradiated group at 14 days after irradiation showing partial improvement of hepatocyte around the central vein (CV) and dilated sinusoid (DS). (H & E ×200).

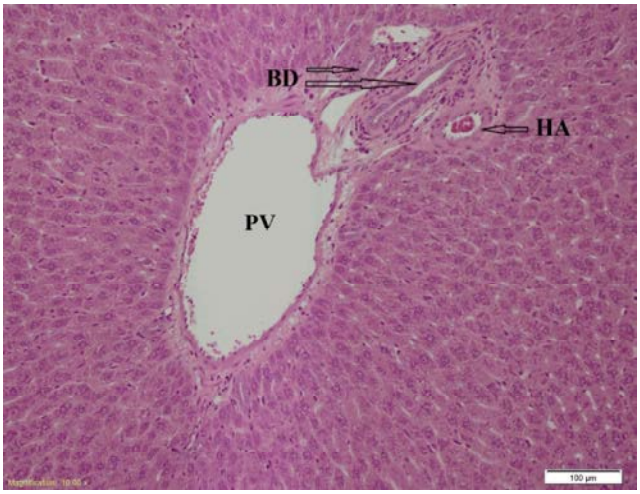

Fig. 10: Light micrograph of rat liver section from pre-treated irradiated group at 1 day after irradiation showing portal area Including hepatic artery (HA), hyalinization area around portal vein (PV) and proliferation of bile ductless (BD). (H & E ×200)

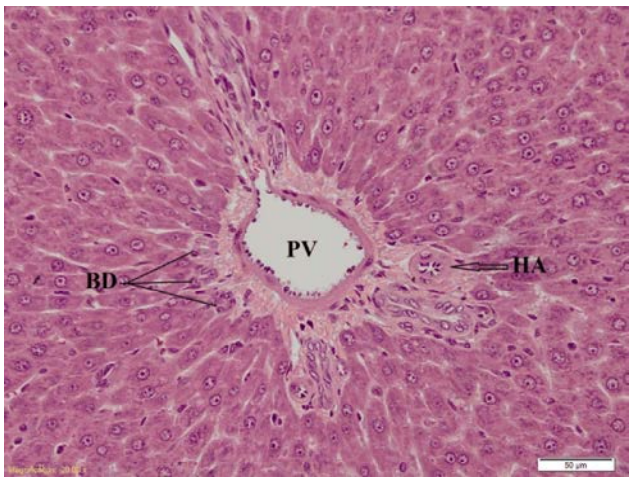

Fig. 11: Light micrograph of rat liver section from pre-treated irradiated group at 14 days after irradiation showing partial improvement of hepatocyte, hyalinization area around portal vein (PV), hepatic artery (HA) and proliferation of bile ductless (BD). (H & E ×400)

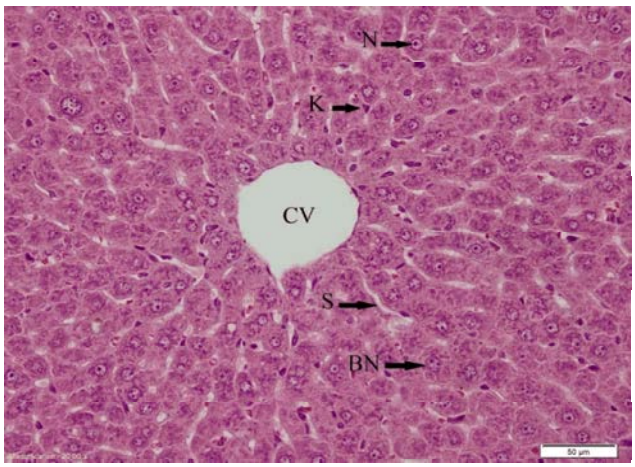

Fig. 12: Light micrograph of rat liver section from pre and post-treated irradiated group at 14 days after irradiation showing recovered hepatic area, hepatocytes regained their normal organization around the central vein (CV) with their normal nuclei (N), binucleated (BN), Kupffer cell (K) and clear hepatic sinusoids (S). (H & E ×400).

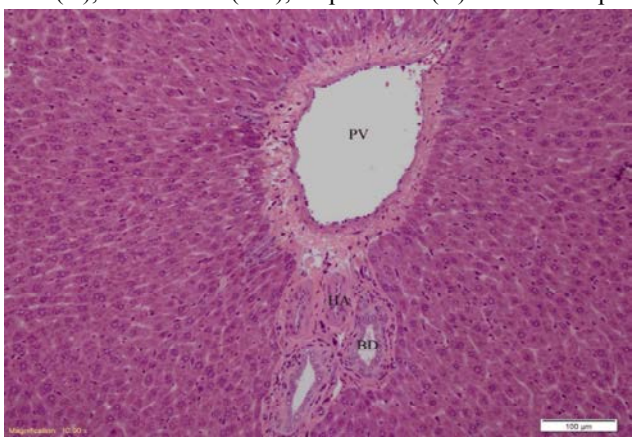

Fig. 13: Light micrograph of rat liver section from pre and post-treated irradiated group at 14 days after irradiation showing normal hepatocyte, hyalinization area around portal vein (PV)), hepatic artery (HA) and proliferation of bile ductless (BD). (H & E ×200)

Pre-treated irradiated group after one-day post irradiation (Figs. 8-11) showing moderate changes. At 14 days post irradiation showed partially improvement of structural changes and hepatocyte, which exhibited reduced centrilobular necrosis in addition to near normal sinusoidal spaces. In pre and post-treated irradiated group at 14 days post irradiation, the central vein appears with a normal pattern. The hepatocytes were almost in normal shapes and regained their normal organization and architecture (treatment brought back the cellular arrangement around the central vein) and cell necrosis was not obvious. While, proliferation of bile ductless and dilatation of portal vein were still found (Figs. 12 and 13). The pre and post-treated irradiated group were also characterized by an increased number of bi-nucleated cells.

DISCUSSION

The significant increase in the liver relative weight after one-day post-irradiation might explained by the acute damage affecting the liver post- irradiation. Acute hepatic toxicity from completely liver radiation usually involves of the sudden onset of an enlarged liver and ascites [26]. The results are in agreement with Kang *et al.* [27]. After 14 days of irradiation, irradiated group showed a significant decrease in liver relative weight, which might be due to the chronic damage caused by radiation. In the chronic stage, the liver becomes contracted, fibrotic and loses of its volume [26]. The previous results are in agreement with those obtained by Amin *et al.* [28]. On contrary, treatment rats by fenugreek seeds succeeded to prevent liver weight from reduction after 14 days post irradiation. These results are in agreement with those reported by Belaïd-Nouira *et al.* [29], who found that Co-administration of fenugreek seeds powder (FSP) at 5% in pellet diet during two months succeeded to antagonize the hepatotoxicity effects of aluminum chloride ($AlCl_3$) and prevented significantly liver weight loss.

The spleen index is an important marker to monitor damage in the hematopoietic system [30]. The results showed that the ionizing radiation formed alteration in the liver enzymes AST and ALT, which have been widely utilized in mammalian toxicology as biomarkers of specific organ dysfunction [31]. In general¡ the increase of transaminases activity is usually associated with hepatocyte damage. The marked elevation in ALT and AST levels after exposure to radiation might be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage [31, 32-35]. Elevation levels of liver enzymes might be as a result of the damage in cellular membranes of hepatocytes, which leads to increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells to the blood streams [36]. Leading to increase in the aminotransferase activities in liver and blood serum [37] or mitochondrial membrane [38] causing the release of intracellular enzymes into circulation. In addition, Geraci and Mariano [39] recorded that the leakage of AST from liver slices in vitro correlated with the AST leakage from irradiated liver into the plasma *in vivo*, indicating hepatocyte membrane damage induced by irradiation. The destruction in liver cells also could refer to the increments in lipid peroxidation and the depression in antioxidant defense in liver cells.

In the present study, results showed that fenugreek seeds decreased the deleterious effect of radiation on liver enzymes and protecting liver activity. Kaviarasan and Anuradha [40] examined the hepatoprotective effect of fenugreek seeds extract *in vivo* using chronic ethanol-induced hepatic injury in a rat model. Fenugreek seeds extract administration restored the altered levels of liver function enzymes (AST, ALT and ALP). Various studies demonstrated the protective effects of fenugreek seeds against various factors-induced liver injury by normalizing the markers of hepatic injury [41-44], which could be attributed to the protective effect of fenugreek seeds on hepatic tissues [45[. Thiol groups are essential in cellular structure and function maintenance [46]. Endogenous cellular thiol-dependent enzymes and non-enzymatic antioxidant such as non-protein thiols consist of glutathione (GSH) and low molecular weight species such as cysteine, cysteamine and coenzyme A contents playing an important role in radiation response. They are good scavenger for reactive oxygen species (ROS) and participates in a wide range of cellular functions [47]. The exogenous addition of GSH can effectively reduce radiation-induced micronuclei [48] chromosome aberrations [49] in mammalian cells. Likewise, depletion of endogenous GSH by buthionine sulfoximine (GSH-depleting agent) increases DNA damage induced by ionizing radiation in mammalian cells [50].

In the present study, a high significant decrease in total thiols content was observed in whole-body irradiated rats. This decrease could be attributed to radiation attack sulfhydryl groups (-SH groups) of proteins and the level of blood and liver glutathione [51]. These groups belong to the most sensitive configuration, which becomes oxidized by ionizing radiation [52].

In irradiated rats group treated with fenugreek seeds, total thiols content were significantly increase comparing to irradiated rats positive control group. This is might because of high content of thiols in fenugreek. Fenugreek showed a different levels of thiols included glutathione (GSH), cysteine (CYS), homocysteine (HCYS) and γ-glutamyl cysteine (GGC). The results also showed that thiol status is a critical factor in cell survival after irradiation [53]. Under normal conditions, the inherent defense system, including glutathione and antioxidant enzymes protects against the oxidative damage [54]. The intracellular content of glutathione is a responsive to environmental factors and a function of the balance between use and synthesis. Thus, oxidative stress in vivo mainly translates into a deficiency of GSH and/or its precursor, cysteine [55, 56]. The concentration of intracellular GSH, is the key determinant of the extent of radiation-induced hepatic injury [31]. Maintenance of the cellular GSH, a free radical scavenger, is critical for keeping a check on cellular homeostasis. The GSH/GST detoxification system is a important part of cellular defense against a large array of injurious agents. GSH offers protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation [57]. The decrease in reduced glutathione by irradiation could be due to oxidation of the sulfhydryl group of GSH due to the decrease in glutathione reductase and the enzyme, which reduces the oxidized glutathione (GSSG) into a reduced form [58]. The *in vitro* and in-vivo depletion of GSH is known to cause an inhibition of the glutathione peroxidase activity and has been shown to increase lipid peroxidation [59].

Lipid peroxidation is a highly destructive process altering the structure and function of cell membranes [60]. Cellular biomolecules are altered directly by radiation or damaged indirectly by free radical production. Reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical and hydrogen peroxide, either initiates lipid peroxidation by extracting a hydrogen atom from unsaturated membrane lipids [61] or triggers a chain of peroxidation reactions by reacting with the sulfhydryl compounds, that leads to cell injury and other chronic complications [62]. Resulting in the formation of malondialdehyde (MDA), the increase level of MDA in the irradiated group demonstrates the role of oxidative mechanisms in the irradiated rats induced tissue damage. Due to high cytotoxicity and inhibitory actions on protective enzymes, MDA acts as a tumor promoter and a co-carcinogenic agent [63]. These lipid peroxidations can cause severe impairment of membrane function through increasing membrane permeability and membrane protein oxidation [64]. Moreover, the enhanced levels of lipid peroxidation induced by radiation are accompanied by a decrease in the activities of SOD, CAT and GPx [65]. One possible mechanism to explain why GSH content and SOD activity dropped after radiation is because of the degraded or saturated of these compounds to block radiation-induced massive free radical production [66]. Another possibility referred to an enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation [67]. Superoxide dismutase (SODs) is belonging to a ubiquitous family of enzymes that function to efficiently catalyze the dismutation of superoxide anions [68]. Therefore, a reduction in the activity of this enzyme can result in a number of delirious effects due to the accumulation of superoxide radicals and H_2O_2 [61]. The decline in the activities of these antioxidants could also be explained by the fact that excess superoxide radicals may inactivate H_2O_2 scavengers, thus resulting in the inactivation of SOD [69].

The results of the present study demonstrated that whole-body exposed to ionizing irradiation causes oxidative tissue damage in rats liver, as demonstrated by increased lipid peroxidation (LPO) and decrease in antioxidants activities of (enzymatic and non-enzymatic) including antioxidant molecular levels (GSH) and antioxidant enzyme activity (SOD) in liver tissues. They recorded a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides after whole body irradiation. These results are in agreement with those reported by Kunwar *et al.* [30], Anees *et al.* [31], Cihan *et al.* [34], Nwozo *et al.* [35], Verma *et al.* [70], Zhao *et al.* [71], Ran *et al.* [72] and Srinivasan *et al.* [73]. Evidence showed that fenugreek seeds could improve GSH-cycle enzymes and protect the cells from irradiation-induced cell death. Treatment with fenugreek seeds caused significant reduction in lipid peroxides levels and elevation in antioxidant enzyme (SOD) and non-enzymatic antioxidant (GSH). Fenugreek seeds was able to protect liver from damaging effect of radiation. Fenugreek seeds able to prevent from lipid peroxidation and restoration of GSH and SOD in various causes of oxidative stress [45, 74, 75, 76]. It is likely that lipid peroxidation in the liver is owing to antiradical and antioxidant potential of fenugreek seeds emphasized through *in vitro* and *in vivo* experiments [29, 44, 62, 77] Shang *et al.* [78] identified five different flavonoids namely vitexin, tricin, naringenin, quercetin and tricin-7-O-β-d-glucopyranoside to be present in fenugreek seeds. The scavenging activities of the phenolic substances are

attributed to the active hydrogen-donating ability of the hydroxyl substitutions [79]. Recently quercetin, one of the identified flavonoids in fenugreek seeds, was found able to protect rat hepatocytes against oxidative damage induced by ethanol [80]. Liver and the spleen have been reported to be highly radiosensitive hematopoietic organs. The liver is the primary organ responsible for drug metabolism, detoxifying damaging electrophiles generated during oxidative stress and the spleen is the main organ involved in the development of the immune response and functions related to blood such as filters, stores, produces, modifies, destroys and protects the blood elements [81, 82, 83]. In the present study, radiation had adverse effects on the liver and spleen.

Histologically, there was severe liver damage in irradiated rats including hepatocyte edema, focal necrosis, pyknotic nuclei, karyolysis and proliferation of kupffer cells in association with the dilatation and congestion of the central and portal veins and infiltration of the parenchyma with inflammatory cells. These observations are in agreement with those obtained by Ran *et al.* [72], Qi *et al.* [84], Kalpana *et al.* [85] and Xu *et al.* [86]. As for the spleen, the histology of the irradiation rats showed an increase in extramedullary hematopoiesis and a decrease in the lymphocytes in the white pulp. These observations are in agreement with those reported by Ran *et al.* [72] and Xu *et al.* [86]. Pre and post-treated with fenugreek seeds in irradiated group revealed better liver and spleen architecture with significantly regained their normal organization of the hepatocytes and lymphocytes as compared with irradiated rats without treatment, which might be correlated with a lower level of oxidative stress induced by fenugreek seeds treatment, which suggests that fenugreek seeds might promote the hematopoietic functions of the liver and spleen. The ability of fenugreek to prevent pathological changes was already proven in rat hepatocytes against various causes of toxicity [29, 44, 87, 88].

CONCLUSION

Feeding rats with fenugreek seeds for 14 days before and after exposure to irradiation 6.5Gy reduced serum level of total thiols, liver function and endogenous antioxidant status; inhibits the lipid peroxidation in irradiated rats. These effects are associated with amelioration of degenerative histopathological changes in liver and spleen tissues induced by exposure to irradiation. These results suggest that fenugreek mitigated radiation-induced pathological þ þchanges in the liver and spleen in rats. Therefore, fortification of food products with fenugreek seeds or drinking of fenugreek may be beneficial for patients who are plan to exposure to radiation.

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