The effect of gamma irradiation on the quality and natural antioxidants of fenugreek and lupine seeds in hyperlipidemic rats

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Abstract:

The present study aims to evaluate the effect of gamma irradiation on the quality and natural antioxidants of fenugreek (***Trigonella joenum-greacum***) and lupine ***(lupinus terms)*** seeds at dose levels of 10 and 20 kGy in hyperlipidemic rats. Rats were divided into: group (1) fed on balanced diet (negative control), group (2) fed on high fat high cholesterol (HFHC) diet (positive control), groups (3,4,5,6,7 and 8) fed on HFHC diet with either 15% non-irradiated or irradiated at dose levels of 10 or 20 kGy fenugreek or lupine seeds, respectively for eight weeks. The results showed that the applied doses of non-irradiated or irradiated fenugreek or lupine seeds at dose levels of 10 or 20 kGy were significant decrease in relative liver weight except for group (3), PER except for group (4), food intake, final body weight, gain in body weight, serum alanine amino transferase (ALT), serum aspartate amino transferase (AST), total cholesterol, triacylglycerol, low density lipoprotein-cholesterol (LDL-C), malodialdehyde and nitric oxide. On the other hand, it was observed that there were significant increase in FER except for group (5), blood hemoglobin, high density lipoprotein-cholesterol (HDL-C), superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) when compared with HFHC diet (group 2).

Keywords: fenugreek seeds; lupine seeds; HFHC diet; antioxidants; irradiation.

1. Introduction:

Hyperlipidemia is a family of disorders that are characterized by abnormally high levels of lipid in the blood. While lipids play an important role in the body's metabolic processes, high blood levels of lipid increase the risk of cardiovascular disease (CVD) [1].

Cardiovascular disease (CVD) is a growing health concern both in developed countries and in-developing countries resulting from known risk factors such as physical inactivity, overweight, diabetes, hypertension, and hyperlipidemia. Role of food constituents in the prevention of degenerative diseases has received considerable attention. Effective nutritional intervention strategies for preventing or managing CVD also call for basic information on the nutraceutical potential of non-nutrient phytochemicals, due to their antioxidant property and beneficial modulation of lipid homeostasis [2].

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The free radicals can be scavenged by using the antioxidant system including non-enzymatic constituents as (phenols, flavonoides, vitamin C and E) and antioxidant enzymes as (glutathione peroxidase, catalase and superoxide dismutase) which the important antioxidant enzymes are playing a key role in minimizing the oxidative stress. The free radical scavenging activities of medicinal plants considered as the natural antioxidant, which is utilized in several medical applications of effectiveness and safety [3].

Fenugreek (*Trigonella foenum- gracum*) is one of the well-known herbs in human food. Its seeds and green leaves are used in food as well as in medicinal application which is an old practice of human history. Fenugreek, like other legumes, is a good source of dietary protein about 24% for consumption by humans and animals, fatty acids from 5-10% which include linoleic, linolenic, oleic and palmatic. It has 45-65% total carbohydrates with 15% of galactomannose (soluble fiber) and other active compounds required in a human body such as saponins, coumarin and fenugreekine [4].

Bitter lupine (*lupinus terms*) is a good source of nutrients, not only proteins (28-48%) but also lipids (5-10%), dietary fibers (40%), minerals and vitamins. Furthermore, lupine contains phytochemicals with antioxidant capacity such as polyphenol mainly tannins and flavonoids [5]. A protein-rich legume as lupine has been shown to have similar effects of those of soy in lowering serum cholesterol levels [6].

Irradiation is an ecofriendly technology utilized for improving the hygienic quality, nutritional safety and security. The technology involves ionizing radiations which ensured the food safety by extending the shelf life of the wide variety of foods [7]. In addition to the control of microorganisms of foods, ionizing radiation can be used to improve their flavor, palatability and enhance legumes flour protein digestibility. Also increase the bioavailability of nutrients by inactivation of antinutritional factors such as trypsin, tannin and hemagglutinin inhibitors, and it can be used to enhance the antioxidant properties to some extent [8].

2. Material and methods:

2.1.Materials:

- Fenugreek *(Trigonella Foenum-Graecum)* and lupine (*lupinus terms*) seeds were purchased from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.
- Cholesterol and bile salts were purchased from Sigma Chemical Company, (St. Louis, Mo, USA).
- Animal fat (lard) was purchased from the commercial market.

2.1.1. Irradiation treatment:

Fenugreek *(Trigonella Foenum - Graecum)* and lupine *(lupinus terms)* seeds were freed of husk, stone, etc. and were packed in polyethylene bags, and sealed by heat. Each bag contained about 2 kg. They were subjected at ambient temperature to gamma irradiation from ^{60}Co, at National Center for Radiation Research and Technology (*NCRRT*) at Nasr City, Cairo, Egypt. The facility used was Gamma Chamber 400 A, ^{60}Co facility of India. The

applied doses were 10 and 20 kGy delivered at dose rate of 1.606 kGy /h as calibrated using small pieces of radiochromic film [9], at the time of experimentation. The irradiated samples were stored at 5ºC. in refrigerator until used.

2.1.2. Experimental design:

2.1.3.1 Diet:

In the present study the experimental diets used were the balance diet which was prepared as described by *Revees et al.*, [10] and high-fat high-cholesterol diet (HFHC) diet which was prepared as described by *Lin-Lee et al*., [11].

2.1.3.2. Animals:

Male albino rats, Sprague-Dawley strain, with an initial weight of about 75 ±5 g were used. They were obtained from animal house of National Center for Radiation Research and Technology. Atomic Energy Authority (*NCRRT)*, Nasr City, Cairo, Egypt.

2.1.3.3.Experimental design:

The animals were housed individually in plastic cages with wire mesh bottoms at a room temperature of 22 ± 5 ºC and 60 ± 5% relative humidity, with a photoperiod of 12/12 h and water was provided *adlibitum* for eight weeks. Groups of ten rats each were then assigned to receive one of eight experimental diets (i.e. balanced, high fat high cholesterol (HFHC), high fat high cholesterol (HFHC) with either non irradiated or irradiated fenugreek or lupine seeds at dose levels (10 or 20 kGy) diets were presented as follows:

Group (1): Rats fed on balanced diet according to *Revees et al*., [10] and served as negative control.

Group (2): Rats fed high fat high cholesterol (HFHC) diet (15% saturated fat + 0.5 % cholesterol) according to *lin – lee et al*., [11] and served as positive control.

Group (3): Rats fed HFHC diet containing 15 % non- irradiated fenugreek seeds.

Group (4): Rats fed HFHC diet containing 15 % irradiated fenugreek seeds at 10 kGy.

Group (5): Rats fed HFHC diet containing 15 % irradiated fenugreek seeds at 20 kGy.

Group (6): Rats fed HFHC diet containing 15 % non- irradiated lupine seeds.

Group (7): Rats fed HFHC containing 15 % irradiated lupine seeds at 10 kGy.

Group (8): Rats fed HFHC diet containing 15 % irradiated lupine seeds at 20 kGy.

2.2. Methods:

2. 2. 1. Blood and serum samples collection:

At the end of the experimental period (8 weeks), the animals were anesthetized with diethyl ether after 12 hours fasting and the blood was obtained by cardiac puncture using a 20 gauge needle. Blood samples were collected in two sterile test tubes, the first tube containing

ethylene diamine-tetracetic acid (EDTA) sodium salt, as anticoagulant (20 mg/10 ml of blood). The other tube of blood samples were kept for about half a hr. at room temperature to allow blood to clot, then the tubes was centrifuged at 5000 rpm for 15 min and the clear serum was separated and stored in the deep freezer at -10º C for subsequent biochemical analysis.

2. 2. 2. Organs weight:

The determination of organs weight was used as a good indicator of the general health status of the animal fed with feed ingredients treated with ionizing radiation.

The different body organs (liver, heart, kidney, spleen and testes) were removed, blotted to dryness then weighted to the nearest milligram and stored at -20 ºC for subsequent biochemical analysis.

2.2.3. Biological assessment:

- Determination of food intake, initial body weight, final body weight, gain in body weight and organs weight.
- Determination of protein efficiency ratio (PER) according to *Hacker,* [12] and feed efficiency ratio (FER) according to *Hasan et al.,* [13].

2. 2. 4. Biochemical analysis in serum:

- Blood hemoglobin (Hb) was estimated according to *Young,* [14].
- Serum ALT and AST activities were estimated according to *Young,* [15]
- Serum cholesterol, triacylglyserol, HDL-C and LDL-C were estimated according to *Young*, [16], *Stein*, [17], *NCEPR*, [18] and *Friedwald et al.,* [19], respectively.
- Superoxide dismutase activity (SOD), Glutathione peroxidase activity (GPX) and Catalase activity (CAT) were determined in liver tissues by (Biodiagnostic Kit) according to *Minami and Yoshikana,* [20], *Paglia* and *Valentine* [21] and *Aebi*, [22], respectively**.**
- Malondialdehyde (MDA**)** and Nitric oxide (NO) were determined in liver tissues by (Biodiagnostic Kit) according to *Montgomry and Dymock*, [23] and *Ohkawa et al*., [24], respectively.

2. 2. 9. Statistical analysis

The data were subjected to analysis of variance (ANOVA) with one-way classification. Linear regression analysis was utilized to define the relationship between different parameters and irradiation dose (kGy). All analyses were conducted using the general linear model procedure of the Statistical Analysis System Institute (SAS, 2003), where appropriate treatment means was separated using the *Duncan's* Multiple Range Test [25]. Means with the same letter in column are not significantly affected at 5 % level of cofedence ($P \geq 0.05$).

3. Results and Discussion:

3.1. Results:

Organ weight is a good indicator of the general health status of the animals fed with ingredients treated with ionized radiation.

As shown in table (1) there were no significant difference ($P \geq 0.05$) in relative heart, spleen, kidney and testis weights in all applied doses except for liver which significantly deceased in groups (4 and 5) when compared with group (2). Meanwhile there was significant increase in group (2) when compared with group (1).

Table (1): Effect of non-irradiated or irradiated fenugreek seeds at 10 or 20 kGy dose levels on relative organs weight in rats fed HFHCdeit.

Treatments	Liver (g/100g)	Heart (g/100g)	Spleen (g/100g)	Kidney (g/100g)	Testis (g/100g)
Group (1)	$3.70^{b} \pm 0.27$	$0.43^{a} \pm 0.05$	$0.39^{a} \pm 0.04$	$0.79^{a} \pm 0.05$	$1.68^{a} \pm 0.17$
Group (2)	$4.66^{a} \pm 0.11$	$0.42^{a} \pm 0.02$	$0.38^{a} \pm 0.01$	$0.78^{a} \pm 0.10$	$1.75^{a} \pm 0.23$
Group (3)	$4.67^{a} \pm 0.10$	$0.33^{a} \pm 0.03$	$0.35^{a} \pm 0.02$	$0.76^{a} \pm 0.15$	$1.45^{a} \pm 0.12$
Group (4)	$3.67^{b} \pm 0.22$	$0.42^{a} \pm 0.03$	$0.42^{a} \pm 0.03$	$0.80^{a} \pm 0.10$	$1.58^{a} \pm 0.25$
Group (5)	$4.07^{b} \pm 0.08$	$0.39^{a} \pm 0.08$	$0.40^{a} \pm 0.05$	$0.76^{a} \pm 0.04$	$1.40^{a} \pm 0.08$

- **Group (1):** rats fed balanced diet, **Group (2):** rats fed high fat high cholesterol diet (HFHC), **Group (3):** rats fed high fat high cholesterol diet (HFHC) with none irradiated fenugreek seeds, **Group (4):** rats fed high fat high cholesterol diet (HFHC) with irradiated fenugreek at 10 kGy, **Group (5):** rats fed high fat high cholesterol diet (HFHC) with irradiated fenugreek at 20 kGy.
- Results presented as mean ± SE. - Means with the same letter in column are not significantly affected at 5 % level of cofedence ($P \geq 0.05$).

Furthermore, table (2) presented that there were no significant difference ($P \geq 0.05$) in relative heart, spleen, kidney and testis weights in all applied doses except for liver which significantly deceased ($P \leq 0.05$) in groups (6, 7 and 8) when compared with group (2).

Table (2): Effect of non-irradiated or irradiated lupine seeds at 10 or 20 kGy dose levels on relative organs weight in rats fed HFHC diet.

Treatments	Liver (g/100g)	Heart (g/100g)	Spleen (g/100g)	Kidney (g/100g)	Testis (g/100g)
Group (1)	$3.70^{c} \pm 0.01$	$0.43^{a} \pm 0.05$	$0.39^{a} \pm 0.04$	$0.79^{a} \pm 0.05$	$1.68^{a} \pm 0.17$
Group (2)	$4.66^{a} \pm 0.01$	$0.42^{a} \pm 0.02$	$0.38^{a} \pm 0.01$	$0.78^{a} \pm 0.10$	$1.75^{a} \pm 0.05$
Group (6)	$4.05^{b} \pm 0.02$	$0.48^{a} \pm 0.03$	$0.41^{a} \pm 0.06$	$1.07^{a} \pm 0.06$	$1.73^{a} \pm 0.02$
Group (7)	$3.94^{b} \pm 0.03$	$0.49^{a} \pm 0.03$	$0.40^{a} \pm 0.06$	$1.05^{a} \pm 0.07$	$1.77^{a} \pm 0.04$
Group (8)	$3.55^{d} \pm 0.04$	$0.40^{a} \pm 0.03$	$0.43^{a} \pm 0.05$	$1.09^{a} \pm 0.04$	$1.76^{a} \pm 0.02$

- **Group (1):** rats fed balanced diet, **Group (2):** rats fed high fat high cholesterol diet (HFHC), **Group (6):** rats fed high fat high cholesterol diet (HFHC) with none irradiated lupine seeds, **Group (7):** rats fed high fat high cholesterol diet (HFHC) with irradiated lupine at 10 kGy, **Group (8):** rats fed high fat high cholesterol diet (HFHC) with

irradiated lupine at 20 kGy. – Results presented as mean ± SE. - Means with the same letter in column are not significantly affected at 5 % level of cofedence ($P \geq 0.05$).

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Analysis of PER presented in table (3) were significantly decreased in groups (3and 5) when compared with group (2). Meanwhile, there was no significant change between group (4) and group (2).

FER were significantly increased in groups (3 and 4) when compared with group (2). Meanwhile, there were no significant change between group (5) and group (2).

Table (3): Effect of non-irradiated or irradiated fenugreek seeds at 10 or 20 kGy dose levels on Protein efficiency ratio (PER) and feed efficiency ratio (FER) in rats fed HFHC diet.

Treatments	PER (g)	FER (g)
Group (1)	9.49^{b}±0.41	5.48^{c}± 0.01
Group (2)	10.62^{a}±0.04	6.79^{b}± 0.03
Group (3)	9.56^{b}±0.78	7.23^{a}± 0.02
Group (4)	10.37^{a}±0.03	7.69^{a}± 0.01
Group (5)	8.87^{b}±0.20	6.94^{b}± 0.01

- **PER:** protein efficiency ratio and **FER:** feed efficiency ratio. - Legends as in table (1).

Results in table (4) indicated that analysis of PER were significantly decreased in groups (6, 7 and 8) when compared with group (2). While FER were significantly increased in groups (6, 7 and 8) when compared with group (2).

Table (4): Effect of non-irradiated or irradiated lupine seeds at 10 or 20 kGy dose levels on Protein efficiency ratio (PER) and feed efficiency ratio (FER) in rats fed HFHC diet.

Treatments	PER (g)	FER (g)
Group (1)	9.49^{c}±0.41	5.48^{c}± 0.01
Group (2)	10.62^{a}±0.04	6.79^{b}± 0.03
Group (6)	9.46^{bc}± 0.03	7.09^{a}± 0.01
Group (7)	10.38^{b}± 0.03	7.91^{a}± 0.08
Group (8)	10.34^{b}± 0.05	8.02^{a}± 0.03

PER: protein efficiency ratio and **FER:** feed efficiency ratio. - Legends as in table (2).

Table (5) presented a significant decrease in food intake, final body weight and gain in body weight of groups (3, 4 and 5) when compared with group (2). Also there were a significant decreased in final body weight and gain in body weight in group (5) when compared with groups (3 and 4).

Table (5): Effect of non-irradiated or irradiated fenugreek seeds at 10 or 20 kGy dose levels on food intake, initial body weight, final body weight and gain in body weight in rats fed HFHC diet.

Treatments	Food intake (g/day)	Initial body weight (g)	Final body weight (g)	Gain in body weight(g)
Group (1)	17.31^{a}±0.08	75.17^{a}± 0.01	170.07^{b}± 0.57	94.90^{b}±0.78
Group (2)	15.64^{b}±0.12	79.40^{a}± 0.02	185.66^{a}± 0.21	106.26^{a}±0.08
Group (3)	13.23^{c}±0.15	80.61^{a}± 0.05	176.30^{b}± 0.34	95.69^{b}±0.70
Group (4)	13.02^{c}±0.39	75.20^{a}± 0.04	178.91^{b}± 0.67	103.71^{b}±0.71
Group (5)	12.77^{c}±0. 46	70.31^{a}± 0.03	159.05^{c}±0.57	88.74^{c}±0.32

– Legends as in table (1).

Table (6) illustrated a significant decrease in food intake, final body weight and gain in body weight of groups (6), (7) and (8) when compared with group (2). Also a significant decreased in final body weight of group (6) when compared with groups (7) and (8).

Table (6): Effect of non-irradiated or irradiated lupine seeds at 10 or 20 kGy dose levels on food intake, initial body weight, final body weight and gain in body weight in rats fed HFHC diet.

Treatments	Food intake (g/day)	Initial body weight (g)	Final body weight (g)	Gain in body weight (g)
Group (1)	17.31^{a}±0.08	75.17^{a}± 0.01	170.07^{b}± 0.57	94.90^{b}±0.78
Group (2)	15.64^{b}±0.12	79.40^{a}± 0.02	185.66^{a}± 0.21	106.26^{a}±0.08
Group (6)	13.33^{c}±0.15	73.75^{a}± 1.70	168.38^{c}± 8.33	94.63^{b}±15.17
Group (7)	13.12^{c}±0.39	77.16^{a}± 1.70	180.96^{b}± 8.33	103.80^{b}±15.17
Group (8)	12.88^{c}±0. 46	74.46^{a}± 1.70	177.88^{b}± 8.33	103.42^{b}±15.17

– Legends as in table (2).

Table (7) showed a significant increase in hemoglobin value of groups (3, 4 and 5) when compared with group (2). Although, there was no significant different ($P \leq 0.05$) between groups (3, 4 and 5) when compared with group (1).

Table (7): Effect of non-irradiated or irradiated fenugreek seeds at 10 or 20 kGy dose levels on hemoglobin values in rats fed HFHC diet.

Treatments	Hemoglobin (g/dL)
Group (1)	$14.75^{a} \pm 0.07$
Group (2)	$12.51^{b} \pm 0.03$
Group (3)	$14.28^{a} \pm 0.15$
Group (4)	$14.60^{a} \pm 0.30$
Group (5)	$14.23^{a} \pm 0.14$

– Legends as in table (1).

Table (8) explained that there was a significant increase in hemoglobin value of groups (6), (7) and (8) when compared with group (2). On the other hand, there was no significant different between groups (6), (7) and (8) when compared with group (1).

Table (8): Effect of non-irradiated or irradiated lupine seeds at 10 or 20 kGy dose levels on hemoglobin values in rats fed HFHC diet.

Treatments	Hemoglobin (g/dL)
Group (1)	$14.75^{a} \pm 0.05$
Group (2)	$12.51^{b} \pm 0.08$
Group (6)	$13.86^{a} \pm 0.0$١
Group (7)	$14.09^{a} \pm 0.02$
Group (8)	$15.30^{a} \pm 0.05$

– Legends as in table (2).

Table (9) showed that there were a significant ($P \leq 0.05$) decrease in both serum ALT and AST of groups (3, 4 and 5) when compared with group (2). Group (5) which was provided with highest dose of fenugreek at 20 kGy showed a significant ($P \leq 0.05$) decrease in serum ALT and AST when compared with group (3 and 4).

Table (٩): Effect of non-irradiated or irradiated fenugreek seeds at 10 or 20 kGy dose levels on serum ALT and AST activities in rats fed HFHC diet.

Treatments	ALT (U/L)	AST (U/L)
Group (1)	$12.96^{c} \pm 0.09$	$32.48^{b} \pm 0.11$
Group (2)	$15.64^{a} \pm 0.46$	$33.26^{a} \pm 0.11$
Group (3)	$13.92^{b} \pm 0.05$	$32.89^{b} \pm 0.23$
Group (4)	١٣.٤٤$^{b} \pm 0.14$	$32.03^{b} \pm 0.30$
Group (5)	$12.80^{c} \pm 0.28$	$31.00^{c} \pm 0.69$

– Legends as in table (1).

Table (10) indicated that there were a significant decrease in both serum ALT and AST activities of groups (6, 7 and 8) when compared with group (2).

Table (10): Effect of non-irradiated or irradiated lupine seeds at 10 or 20 kGy dose levels on serum ALT and AST activities in rats fed HFHC diet.

Treatments	ALT (U/L)	AST (U/L)
Group (1)	13.92^{b}± 0.09	32.48^{b}± 0.11
Group (2)	14.82^{a}± 0.46	33.17^{a}± 0.11
Group (6)	12.88^{c}± 0.08	32.37^{b}± 0.34
Group (7)	13.17^{cb}± 0.01	30.99^{c}± 0.39
Group (8)	13.97^{b}± 0.05	32.14^{b}± 0.11

– Legends as in table (2).

Results in table (11) showed a significant decrease in serum total cholesterol, triacylglycerol and LDL-C in groups (3, 4 and 5) when compared with group (2). Meanwhile, there was a significant ($P \leq 0.05$) increase in HDL-C in groups (3, 4 and 5) when compared with group (2).

The mean value of serum cholesterol in groups (4 and 5) return to normal levels as in group (1). The high irradiation dose level of fenugreek seeds at 20 kGy (group 5) showed a significant decrease in triacylglycerol when compared with groups (3 and 4).

Table (11). Effect of non-irradiated or irradiated fenugreek seeds at 10 or 20 kGy dose levels on serum total cholesterol, triacylglycerol, HDL-C and LDL-C in rats fed HFHC diet.

Treatments	Cholesterol (mg/dL)	Triacylglycerol (mg/dL)	HDL- C (mg/dL)	LDL – C (mg/dL)
Group (1)	175.58^{c} ±0.66	80.42^{d} ± 1.52	56.61^{b} ± 0.56	102.88^{b} ± 0.79
Group (2)	211.59^{a} ± 0.65	183.27^{a} ± 0.77	54.15^{c} ± 0.95	120.79^{a} ± 1.16
Group (3)	179.43^{b} ± 4.17	127.84^{b} ± 0.74	57.00^{a} ± 0.71	96.87^{c} ± 4.80
Group (4)	177.11^{c} ± 1.17	127.52^{b} ± 0.68	58.05^{a} ± 0.58	93.56^{c} ± 1.18
Group (5)	177.07^{c} ± 1.84	125.11^{c} ± 17.01	58.51^{a} ± 0.30	93.54^{c} ± 1.85

- **HDL-C:** high density lipoprotein- cholesterol, **LDL-C:** low density lipoprotein-cholesterol. – Legends as in table (1).

Results in table (12) showed a significant decrease in serum total cholesterol, triacylglycerol and LDL-C in groups (6, 7 and 8) when compared with group (2).

Meanwhile, there was a significant increase in HDL-C in groups (6, 7 and 8) when compared with group (2).

The mean value of serum cholesterol in groups (7 and 8) return to normal levels as in group (1). The high irradiation dose level of lupine seeds at 20 kGy (group 8) showed a significant decrease in triacylglycerol when compared with groups (6 and 7).

Table (12). Effect of non-irradiated or irradiated lupine seeds at 10 or 20 kGy dose levels on serum total cholesterol, triacylglycerols, HDL-C and LDL-C in rats fed HFHC diet.

Treatments	Cholesterol (mg/dL)	Triacylglycerol (mg/dL)	HDL- C (mg/dL)	LDL – C (mg/dL)
Group (1)	175.58 ^{c}± 0.66	80.42 ^{d}± 1.52	56.61 ^{b}± 0.56	102.88 ^{b}±0.79
Group (2)	211.59^{a}± 0.65	183.27 ^{a}± 0.77	54.15 ^{c}± 0.95	120.79 ^{a}± 1.16
Group (6)	194.90^{b}± 1.90	116.73 ^{b}± 2.38	57.81 ^{a}± 0.58	97.09 ^{c}± 2.44
Group (7)	179.24^{c}± 1.15	113.29 ^{b}± 1.59	57.19 ^{a}± 0.50	79.39 ^{c}± 0.46
Group (8)	179.69^{c}± 6.47	100.0 ^{c}± 3.47	57.41 ^{a}± 1.19	78.94 ^{c}± 6.09

- **HDL-C:** high density lipoprotein- cholesterol, **LDL-C:** low density lipoprotein-cholesterol. – Legends as in table (2).

The results in table (13) explained the activity of liver superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) enzymes which were significantly ($P \leq 0.05$) increased in groups (3, 4 and 5) when compared with group (2). The activity of liver SOD was significantly increased in group (5) when compared with groups (3 and 4). The activity of liver GPX was significantly increased in groups (4 and 5) when compared with group (3). While there were no significant differences ($P \geq 0.05$) in liver CAT activity of groups (3, 4 and 5).

Table (13). Effect of non-irradiated or irradiated fenugreek seeds at 10 or 20 kGy on liver Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase (CAT) activities in rats fed HFHCdiet.

Treatments	SOD (U/gm)	GPX (U/gm)	CAT (U/gm)
Group (1)	239.93^{c}± 17.16	134.01^{b}± 15.13	363.95^{a}± 13.40
Group (2)	220.47^{d}± 36.10	101.59^{c}± 5.72	355.78 ^{b}± 25.61
Group (3)	252.89^{b}± 11.23	136.14^{b}± 12.99	462.58^{a}± 11.80
Group (4)	268.59^{b}± 42.52	169.60^{a}± 1.73	465.99^{a}± 21.15
Group (5)	296.51^{a}± 1.75	175.44^{a}± 2.51	467.01^{a}± 7.05

- **SOD:** superoxide dismutase, **GPX:** Glutathione peroxidase and **CAT:** Catalase.

– Legends as in table (1).

Table (14) illustrated the activity of liver superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) which were significantly increased in groups (6, 7 and 8) when compared with group (2). There were no significant differences ($P \geq 0.05$) in SOD and CAT activities of groups (6, 7 and 8). While the activity of GPX of groups (7 and 8) was significantly increased when compared with group (6).

Table (14). Effect of non-irradiated or irradiated lupine seeds at 10 or 20 kGy on liver Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase (CAT) activities in rats fed HFHC diet.

Treatments	SOD(U/gm)	GPX (U/gm)	CAT(U/gm)
Group (1)	239.93^{a}± 17.16	134.01^{b}± 15.13	363.95^{b}± 13.40
Group (2)	220.47^{b}± 36.10	101.59^{c}± 5.72	355.78^{c}± 25.61
Group (6)	240.03^{a}± 11.23	153.47^{b}± 21.93	425.17^{a}± 11.32
Group (7)	242.66^{a}± 23.38	163.28^{a}± 12.99	440.15^{a}± 28.30
Group (8)	249.99^{a}± 22.46	168.08^{a}± 15.48	489.76^{a}± 21.15

- **SOD:** superoxide dismutase, **GPX:** Glutathione peroxidase and **CAT:** Catalase
- Legends as in table (2).

Table (15) showed a significant decrease in liver malondialdehyde and nitric oxide in group (3, 4 and 5) when compared with group (2). While there were no significant ($P \geq 0.05$) differences between groups (3, 4 and 5) in liver malondialdehyde and nitric oxide.

Table (15). Effect of non-irradiated or irradiated fenugreek seeds at 10 or 20 kGy on liver Malondialdehyde (MDA) and Nitric Oxide (NO) in rats fed HFHC diet.

Treatments	MDA (nmol/gm)	NO (µmol/ gm)
Group (1)	5.00^{b}± 0.00	44.0^{c}± 3.06
Group (2)	7.73^{a}± 1.46	48.23^{a}± 1.72
Group (3)	4.74^{b}± 0.23	47.32^{b}± 4.32
Group (4)	5.06^{b}± 0.24	46.65^{b}± 0.50
Group (5)	4.29^{b}± 0.55	46.09^{b}± 3.53

– **Malondialdehyde:** MDA**, Nitric Oxide:** NO - Legends as in table (1).

The data presented in table (16) showed that there were a significant decrease in liver malondialdehyde and nitric oxide in group (6, 7 and 8) when compared with group (2). While there were no significant ($P \geq 0.05$) differences between groups (6, 7 and 8) in liver malondialdehyde and nitric oxide.

Table (16). Effect of non-irradiated or irradiated lupine seeds at 10 or 20 kGy on liver Malondialdehyde (MDA) and Nitric Oxide (NO) in rats fed HFHCdiet.

Treatments	*Malondialdehyde (µmol/g.T)*	*Nitric Oxide (µmol/g.T)*
Group (1)	$5.00^{c} \pm 0.88$	$44.0^{c} \pm 3.06$
Group (2)	$7.73^{a} \pm 1.46$	$48.23^{a} \pm 1.72$
Group (6)	$5.94^{b} \pm 1.31$	$43.27^{b} \pm 0.15$
Group (7)	$5.54^{b} \pm 1.49$	$42.57^{b} \pm 1.44$
Group (8)	$5.82^{b} \pm 0.44$	$41.91^{b} \pm 7.18$

- **Malondialdehyde:** MDA**, Nitric Oxide:** NO – Legends as in table (2).

3.2. Discussion:

The values of relative liver weight of groups (4, and 5) were significantly decreased when compared with group (2). Also the liver size was decreased in groups (6), (7) and (8) when compared with group (2). This may be explained by the presence of both high fiber content and bioactive compounds (antioxidants) in fenugreek and lupine seeds which agrees with those obtained by *El- Neily and El-Shenawy,* [26]**.**

Analysis of PER were significantly decreased in all applied doses except for group (4). Meanwhile FER were significantly increased in all groups fed fenugreek and lupine seeds except for group (5) when compared to group (2).

Mahmoud et al., [27] who revealed that a significant decrease in FER when rats fed fenugreek seeds supplementation (5%) diets when compared with high fat diet. These effects are reflected by growth inhibition, negative nitrogen balance, reduced intestinal absorption of sugars and amino acids, reduced immune response and increased liver and protein catabolism.

These results showed no significant differences in initial body weight between all groups. Also the results showed a significant decrease in final body weight and gain in body weight in groups (3, 4, 5 6, 7 and 8) compared with group (2).

konopelnyuk et al., [28] illustrated two possible mechanisms of fenugreek decreasing the total body weight. Fenugreek flushes out the carbohydrates from the body before they enter the blood stream resulting in weight loss. Fenugreek seeds contain a high proportion of soluble fiber. This fiber forms a gelatinous structure which may have effects on slowing the digestion and absorption of food from the intestine and creates a sense of fullness in the abdomen, thus suppresses appetite and promotes weight loss.

These results were agreeing with *mahmoud et al.*, [27] who found that the greatest improvement effect on body weight gain where in rats fed raw fenugreek seeds than other experimental groups.

There was significant increase in hemoglobin value of groups (3, 4, 5, 6, 7 and 8) when compared with group (2) but there were no significant different between groups (3, 4, 5, 6, 7 and 8) when compared with group (1).

These results were disagreeing with *Abd-El Rahman*, [29] who found decrease in hemoglobin value in rats fed diet supplemented with 5 % fenugreek seeds when compared with control diet.

The study revealed that the activities of ALT and AST of groups (3, 4, 5, 6, 7 and 8) were significantly decreased when compared with group (2). Group (5) which was provided with highest dose of fenugreek at 20 kGy showed a significant decrease in serum activities of ALT and AST when compared with group (3).

In the present study, there was a significant increased ($p \leq 0.05$) in ALT and AST activities of rats fed the HFHC diet compared to the control. The supplementation of fenugreek in the HFHC diet caused a reduction in these enzyme activity compared to rats fed HFHC diet. Based on this result, fenugreek may have hepatoprotective effect.

The results of the present study seems to be in line with *Murugesan et al*., [30] who demonstrated that, oral administration of fenugreek improved liver function toward normal values in rats that disturbed by isoproterenol. Also, administration of ethanolic extract of Fenugreek decreased ALT and AST activities in streptozotocin-induced diabetic rats.

Eidi et al., [31] indicated that hepatoprotective effect of Fenugreek was supported by histopathological findings which revealed fatty changes in liver of rats fed high cholesterol diet and significant recovery when fenugreek seed powder has been administered.

This experimental study showed that there was a significant decrease in serum total cholesterol, triacylglycerol and LDL-C in groups (3, 4, 5, 6, 7 and 8) when compared with groups (2). Meanwhile, there was a significant ($P \leq 0.05$) increase in HDL-C in groups (3, 4 5, 6, 7 and 8) when compared with group (2).

The mean value of serum cholesterol in groups (4 and 5) return to normal levels as in group (1). The high irradiation dose level of fenugreek seeds at 20 kGy (group 5) showed a significant decrease in triacylglycerol when compared with groups (3 and 4).

The mean value of serum cholesterol in groups (7 and 8) return to normal levels as in group (1). The high irradiation dose level of lupine seeds at 20 kGy (group 8) showed a significant decrease in triacylglycerol when compared with groups (6 and 7).

Chauhan et al., [32], *Belguith-Hadriche et al.*, [33] and *Korish and Arafah*, [34] studied the effect of high cholesterol diet on rat's demonstrated different extract of fenugreek seeds. The results showed that ethyl acetate extract of Fenugreek reduced total cholesterol and LDL-c concentration and increased HDL-c concentration in rats fed high cholesterol diet.

Xue et al., [35] demonstrated that, administration of ethanol and aqueous extracts of fenugreek, respectively decreased triacylglycerol, total cholesterol, LDL-c and increased HDL-c concentrations in diabetic rats. *Elmnan et al.*, [36] also reported that, fenugreek seeds powder reduced total cholesterol and LDL-c concentration and increased HDL-c concentration in dose dependent manner (0.25 - 0.75%) in rats.

In addition, other report *Moosa et al.*, [37] demonstrated that oral administration of 25 g of fenugreek seed produced by significant reduction of total cholesterol and LDL-c without any changes in HDL-c concentration in hypercholesterolemic patients. The hypocholesterolemic effect of fenugreek might be attributed to significant content of saponins which are known to have hypocholesterolemic effect [38]. Therefore, it has been suggested that, saponins prevent the absorption of lipid either directly in the intestine or bind to bile acids during enterohepatic reabsorption mechanism.

The crude fibers contents of fenugreek seeds are one of the most hypocholesterolemic agents because the presence of soluble fiber may block cholesterol absorption from the intestine [36].

It is well known that, protein quality and quantity play an important role on cholesterol levels. Plants proteins decreased the cholesterol level and exert a lipid lowering capacity [39].

On determining the activity of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) in liver. There were significant increase in groups (3, 4 and 5) when compared with group (2). Also the results showed that the level of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) were significantly increased in groups (6, 7 and 8) when compared with group (2).

Belguith-Hadriche et al., [33] found that, the content of catalase and superoxide dismutase (SOD) in liver tissues decreased significantly after oral administration of the ethyl acetate extract of fenugreek seeds, compared with those of rats fed high cholesterol diet. The phenolic and flavonoïd contents were highest in the methanol and the ethyl acetate extracts. These results showed that the ethyl acetate extract of the fenugreek seeds had a significant hypocholesterolemic effect and antioxidant activity in cholesterol-fed rats.

It was found that the liver malondialdehyde and nitric oxide were significantly decreased in groups (3, 4, 5, 6, 7 and 8) when compared with groups (2).

It was documented that intake of high fat diet causes abnormal rise in lipid and lipoprotein levels and also increases the lipid peroxidation that may lead to oxidative damage due to the formation of free radicals in the liver. The decrease in lipid peroxidation can reduce the atherosclerosis development due to hyperlipidemia. The increase in formation of malondialdehyde (MOD) in rats fed high fat diet is responsible for the increase in hyperlipidemia process.

Arnoldi et al.,[40] studied the interest for lupine is continuously growing showed it provides useful health benefits. The addition of lupine to the diet of different models of hypercholesterolaemia, such as rat, rabbit, hamster and pig, induces increases of HDL cholesterol and cholesterol lowering activity provided controversial results. Those involving hypercholesterolaemic subjects and based on improved lupine foods gave statistically significant LDL-cholesterol reductions.

Durkar et al., [41] reported that the administration of (*symplocos bark*) to hyperlipidemic rats significantly inhibit malondialdehyde formation in liver. The reduced GSH is one of the most non-enzymatic antioxidant present in liver and other tissues. The decreased GSH in high fat diet may be due to increased utilization of GSH to activities of GPX, SOD and CAT.

4. Conclusion:

Food irradiation is a method of food preservation that may not be familiar to many, but it has been in development since the early decade of the twentieth century. If properly applied, irradiation can be effective way to treat a variety of problems in our food supply, such as insect, sprouting, rapid ripening and bacterial growth. The most effective of irradiated fenugreek and lupine seeds at dose levels of 10 and 20 kGy had antihyperlipidemic and hyercholesteromic effects due to accompanied by a marked decreased in protein efficiency ratio (PER), serum ALT, serum AST, total cholesterol, triacylglycerol, malonaldehyde (MDA) and nitric oxide (NO). As compared with increased in blood hemoglobin, feed efficiency ratio (FER), superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). This resulted from enhance the antioxidants properties and enzyme activities.

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الملخص باللغة العربية

تأثير المعالجة الإشعاعية علي مضادات الاكسدة الطبيعية وجودة بذور الحلبة و الترمس علي الجرذان المصابة بارتفاع دهون الدم

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تهدف هذه الدراسة إلى تقييم تأثير أشعة جاما على مضادات الأكسدة الطبيعية وجودة بذور الحلبة أوالترمس عند مستويات جرعة من ١٠ أو ٢٠ كيلو جراي في الجرذان المصابة بارتفاع دهون الدم. تم تقسيم الجرذان الي: مجموعه (١) مجموعه ضابطه و معطاه الوجبه الاساسيه، مجموعه (٢) مجموعه ضابطه ايجابية ومعطاه وجبه عالية من الدهون و الكوليستيرول، مجموعات (٣، ٤، ٥,٦ ،٧ ،٨) معطاه وجبه عالية من الدهون و الكوليستيرول مضاف اليها أيا من ١٥% اليها بذور الحلبة أو الترمس الغير مشعة أو المشعة عند ١٠ أو ٢٠ kGy لمدة ٨ أسابيع.أظهرت النتائج أن جرعات الحلبه اوالترمس الغيرمشعة أو المشعه عند ١٠ أو ٢٠ كيلو جراي انخفضت انخفاضا نسبيا في وزن الكبد النسبي ما عدا مجموعة (٣) ، معدل كفاءه البروتين (PER) ما عدا مجموعة (٤) ، الطعام المأكول،وزن الجسم النهائي، انزيم الانين امينو ترانسفيريز (ALT) ، انزيم الاسبرتات امينو ترانسفيريز (AST) ، الكوليسترول الكلي ، الدهون الثلاثيه، كوليستيرول الليبوبروتينات منخفض الكثافه (LDL-C)، المالون داي الديهيد(MDA) وأكسيد النيتريك (NO). علي الجانب الاخر ، لوحظ زيادة معنوية في معدل كفاءة الغذاء (FER) ما عدا مجموعة (٥)، هيموجلوبين الدم ،كوليستيرول الليبوبروتينات مرتفع الكثافه (HDL-c)، انزيم السوبر اكسيد ديسميوتيز (SOD) ، جلوتاثيون بيروكسيديز (GPX) و الكاتلاز (CAT) بالمقارنة مع المجموعه (٢) المغذاه علي وجبه عاليه الدهون و الكوليستيرول.