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EFFICIENCY OF BORAGE SEEDS OIL AGAINST GAMMA IRRADIATION-INDUCED HEPATOTOXICITY IN MALE RATS: POSSIBLE ANTIOXIDANT ACTIVITY

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Abstract

Background: Borage (*Borago officinal* L.) is an annual herbaceous plant of great interest because its oil contains a high percentage of γ-linolenic acid (GLA). The present work was carried out to detect fatty acids composition of the oil extracted from borage seeds (BO) and its potential effectiveness against γ-irradiation- induced hepatotoxicity in male rats.
Materials and Methods: GC-MS analysis of fatty acids methyl esters of BO was performed to identify fatty acids composition. Sixty rats were divided into five groups (12 rats each): Control, irradiated; rats were exposed to (6.5 Gy) of whole body γ-radiation, BO (50 mg/kg b.wt), irradiated BO post-treated and irradiated BO prepost-treated. Six rats from each group were sacrificed at two time intervals 7 and 15 days post-irradiation. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) levels, lipids profile, as well as serum and hepatic reduced glutathione (GSH) and lipid peroxide (malondialdehyde) (MDA) levels were assessed. Histopathological examination of liver sections were also carried out.
Results: The results showed that the high contents of BO extracted by cold pressing, were linoleic acid (34.23%) and GLA (24.79%). Also, oral administration of BO significantly improved serum levels of liver enzymes, lipids profile, as well as serum and hepatic GSH and MDA levels ($p<0.001$) as compared with irradiated rats after 15 days post irradiation. Moreover, it exerted marked amelioration against irradiation-induced histopathological changes in liver tissues. The improvement was more pronounced in irradiated BO prepost-treated group than irradiated BO post-treated.
Conclusion: BO has a beneficial role in reducing hepatotoxicity and oxidative stress induced by radiation exposure. Therefore, BO may be used as a beneficial supplement for patients during radiotherapy treatment.

Key words: Borage seeds oil; γ-irradiation; Hepatotoxicity; Antioxidant.

Abbreviations: GLA, γ-linolenic acid; **BO**, Borage oil; **GC-MS**, Gas chromatography-Mass Spectrometry; **MPa**, Megapascal (Unit of pressure); **Gy**, The Gray (derived unit of ionizing radiation dose); **AST**, Aspartate aminotransferase; **ALT**, Alanine aminotransferase; **GGT**, Gamma glutamyl transferase; **GSH**, Reduced glutathione; **TG**, Triacylglycerols; **TC**, Total cholesterol; **LDL-C**, Low density lipoprotein cholesterol; **HDL-C**, High density lipoprotein cholesterol; **MDA**, Lipid peroxide (Malondialdehyde); **ROS**, Reactive oxygen species; **RT**, Retention time.

Introduction

Ionizing radiation generally affects the living tissues by damaging the cell with direct and indirect action; direct process produces damage signal transduction, as well as disruption in the cells, while indirect effect results from its communication with water-soluble molecules and production of free radicals and their subsequent action on sub-cellular substrates that destroy the structure of cells (Devasagayam et al., 2004; Ebadi, 2007). Ionizing radiation-induces damage in hepatic tissues, as it is one of the most radiosensitive organs (Widmaier et al., 2004). Excess reactive oxygen species (ROS) damage cellular components and cause lesions in an organism. Activated oxygen, however, readily reacts with biomembrane polyunsaturated fatty acids to generated lipid peroxidation, which disturbs physiological functions of living cells (Sardesai, 1995).

Cells show an elaborate defense system to destroy these ROS, which are the antioxidant defenses. The imbalance between oxidant/antioxidants is leading to an excessive production of oxygen metabolites, which creates an oxidative stress (Ho et al., 1998). Antioxidants have the capacity to reduce harmful effects of radiation on normal tissues (Okunieff et al., 2008). Antioxidants supplements reduce the treatment-related adverse effects, which occur during radiation therapy by overcome the oxidative damage to normal cells (Lawenda et al., 2008).

The use of traditional plant-based preparations in remedial therapeutic and preventive medicines has been developed. Borage (*Borago officinalis* L.) from *Boraginaceae* family is an annual herbaceous plant with nutritional

value used in traditional medicine and culinary uses in some countries (Asadi-Samani et al., 2014; Ghahremanitamadon et al., 2014). Borage seeds oil contains mainly triacylglycerol (95%) consisting of C16-C20 fatty acids, while 5% presents minor components composed of tocopherols, flavonoids, phospholipids, sterols, free fatty acids and also mono-and diacylglycerols (Kotnik et al., 2006). Several classes of these components act as antioxidants (Shahidi and Shukla, 1996). Borage seeds oil is the richest plant source of γ-linolenic acid (GLA) which is used as dietary supplement for prevention and/or treatment of various degenerative pathologies illnesses (Asadi-Samani et al., 2014), as osteoporosis (Kruger et al., 1998), diabetes (Das, 2010), and cancer (Ge et al., 2009 ; Itoh et al., 2010). GLA has also been reported to suppress tumor growth *in vitro* (Pham et al., 2006), improve oxygenation status (Mancuso et al., 1997), exert anti-inflammatory activity and display beneficial effects in the early stages of sepsis (Horrobin, 1992; Pontes-Arruda et al., 2011). Therefore, this study aimed to identify and determine fatty acids composition of the oil extracted from borage seeds, as well as to evaluate the potential radioprotective effect of BO against whole body γ-irradiation- induced hepatotoxicity in male rats.

Materials and Methods

Plant Material

Borage seeds (*Borago officinalis L.*) were obtained from Medicinal and Aromatic Plants Research Department, Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture. Seeds were authenticated by Dr. Abdel-Halim Abdel-Mogly Mohamed, Senior researcher of Taxonomy, Flora and Phytotaxonomy Researchers Department, Horticultural Research Institute, Agricultural Research Center, Giza, Egypt.

Animals

Male Albino rats (160-180 g) were obtained from Animal Breeding Unit of the National Research Center (NRC). Rats were kept in standard laboratory conditions. Experiment was done in National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt. Animals were carefully handled in accordance with the guidelines of Committee for Care and Use of Laboratory Animals, National Research Center, Dokki, Egypt.

Radiation Source

Whole body gamma irradiation (6.5 Gy) was performed according to Naguib and Abd El Magaid (2007) at NCRRT, Atomic Energy Authority, Cairo, Egypt, using Gamma Cells-40 biological irradiator furnished with a Caesium-137. The radiation dose rate was 0.61 Gy /min. as calibrated at the time of the experiment.

Extraction of Oil from Borage Seeds

Dry borage seeds (750 g) were crushed using a commercial blender and then subjected to cold pressing. In this method, oil was extracted by continuous screw-pressers (Carver Press, USA) without any chemicals for 20 min at a pressure of 49.0 MPa.

Fatty Acids Composition.
Preparation of Fatty Acids Methyl Esters

Sulphuric acid (30 ml) and absolute methanol (4: 96 V/V) were mixed with 0.2 g BO, then heated under reflux for about 3 hours. The methyl esters were extracted thrice with petroleum ether, and washed several times with distilled water till the washings were neutral to phenolphthalein. The combined fatty acids methyl esters layers were dried over anhydrous sodium sulphate and filtered. The ether was then removed using a rotary evaporator and aliquots of the fatty acid methyl esters were analyzed by mass spectrometer (Ludde et al., 1960).

Gas Chromatography-Mass Spectrometry Analysis of Fatty Acids Methyl Esters

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of fatty acids methyl esters of BO was performed according to Adams (1995) with a Finnigan Mat SSQ 7000 gas chromatography coupled to Flame ionization detector (FID). The analysis was conducted under programmed temperature conditions: 140-240 ºC at 5 ºC min-1 in 30 min (detector and injector temperatures of 260 °C), injection volume and mode of 0.4 µL and split (100:1), and nitrogen gas as carrier gas (20 cm min-1). The desired amount of samples was dissolved in 2 mL of hexane before injection. The flow-rate of 1 mL min-1 with isocratic elution of acetonitrile for 34 min. Compounds of the different fatty acids were identified by their retention times (RT) and interpretation of their mass spectra.

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Experimental Design

Rats (n= 60) were divided into five groups (12 rats/group), all groups fed the standard AIN-93 diet (Reeves et al., 1993), and given water *ad libitum*. The following scheme explained the experimental design: **Control group**; rats (non-irradiated and non-treated) received orally 0.5 ml of distilled water with two drops of Tween-80, **Irradiated group (Irrad)**; rats were exposed to single sub-lethal dose (6.5 Gy) of whole body γ-radiation and received orally 0.5 ml of distilled water with two drops of Tween-80, **Borage oil group (BO)**; rats (non-irradiated) received 50 mg /kg b.wt /day BO (Hamed and Wahid, 2015), through an intragastric gavage dissolved in 0.5 ml of distilled water with two drops of Tween-80. **Irradiated BO post-treated group**; rats received the same gavage dosage of BO orally started 3 hours after irradiation and continued daily for 2 weeks. **Irradiated BO prepost-treated group**; rats were administered orally with BO (50 mg /kg b.wt) daily, started one week before irradiation and continued till the end of the experiment (2 weeks after irradiation). Six rats from each group were sacrificed at the interval of 7 and 15 days post irradiation. Serum samples were collected for biochemical analysis and liver samples for biochemical and histopathological examinations.

Assay of Biomarkers of Hepatotoxicity and Lipids Profile

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Reitman and Frankel, 1957), gamma glutamyl transferase (GGT) (Szewczuk et al., 1988), and lipids profile parameters; triacylglycerols (TG) (Fossati and Prencipe, 1982), total cholesterol (TC) (Meiattini et al., 1987), high density lipoprotein cholesterol (HDL-C) (Demacker et al., 1980), and low density lipoprotein cholesterol (LDL-C) (Marchall, 1992) were determined.

Assay of Serum and Hepatic Reduced Glutathione (GSH) and Lipid Peroxide (MDA)

After blood collection, rats of each group were sacrificed under anesthesia, the liver samples were removed immediately, washed twice with ice-cold phosphate buffer saline (PBS) and homogenized in 5 volume of phosphate buffer (pH 8.0, 0.01 M) using a Polytron homogenizer (pt 3100) (five cycles of 10 s at 3000 rpm). Aliquots were prepared according to Arafa et al. (2005), and used for the assessment of GSH and MDA. Serum and hepatic GSH levels were determined according to Jollow et al. (1974). The procedure is based on the reaction of bis-(3-carboxy-4-nitrophenyl) disulfide reagent by SH group to form 2-nitro-5-mercaptobenzoic acid, which has an intense yellow color that was measured spectrophotometrically at 412 nm. Serum and hepatic lipid peroxide levels were measured by the methods of Vashney and Kale (1990). The procedure involved the reaction between malondialdehyde (MDA; end-product of lipid peroxidation) and thiobarbituric acid to yield stable pink chromospheres, which measured spectrophotometrically at 532 nm.

Histopathological Examination

Liver samples of the sacrificed rats were fixed immediately in 10 % neutral formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol. The specimens were sectioned at 4-6 microns thickness and stained with Hematoxylin and Eosin (H&E stain) then examined under ordinary microscope according to Bancroft et al. (1996).

Statistical Analysis

Results were statistically analyzed using SPSS version 22, one-Way ANOVA, Post Hoc, LSD to compare between groups in the same time interval.

Results

Fatty Acids Composition

The identification of individual fatty acids and percentages were carried out by GC-MS and presented in Table (1). The peaks related to different fatty acids at different retention times (RT) were shown in Figure (1), some of these peaks were detected and identified, while others about (3.43%) were unidentified. Results in Table (1) showed that 11 fatty acids were identified and detected in the oil extracted from borage seeds, some of these fatty acids were saturated represented (13.26 % of total fatty acids) as Lauric, palmitic, stearic and arachidic acid, and the most predominant saturated fatty acid was palmitic acid (7.64%). Seven unsaturated fatty acids were detected and identified including palmitoleic, oleic, linoleic, γ-linolenic, brassidic, erucic and nervonic acids, total unsaturated fatty acids represented (83.31 % of total fatty acids composition). The unsaturated linoleic acid represented the majority of total fatty acids composition (34.23%) followed by γ-linolenic (24.79 %) and oleic acid (14.23 %).

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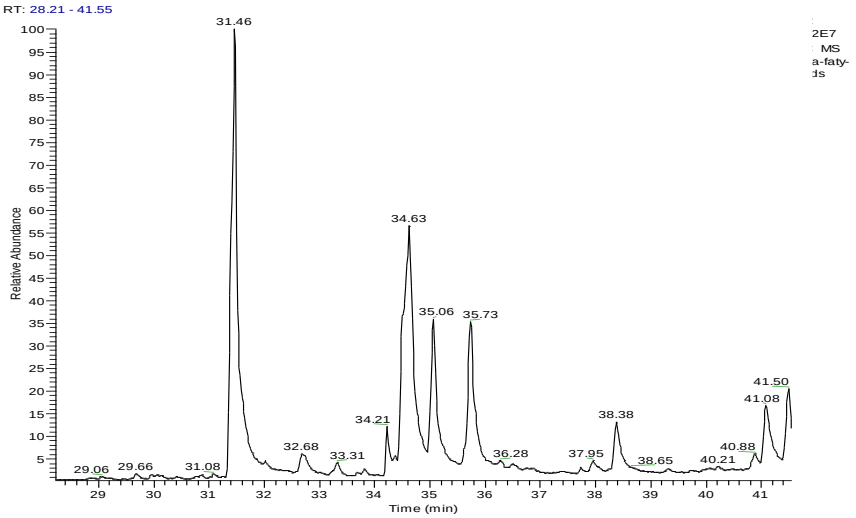

Figure 1: GC-MS of fatty acids methyl esters of oil extracted from borage seeds

Table 1: Fatty acids methyl esters composition of BO as a percentage concentrations of total fatty acids.

Common Name	**RT** (min)	**%**	**Systematic Name**
Lauric acid	29.06	1.14	Dodecanoic acid
Palmitic acid	29.66	7.64	Hexadecanoic acid
Palmitoleic acid *	30.05	6.25	Cis-9- Hexadecanoic acid
U	30.40	1.55	
Stearic acid	30.86	3.08	Octadecanoic acid
Oleic acid *	31.08	14.23	Cis-9-Octadecanoic acid
U	31.46	0.15	
Linoleic acid *	32.68	34.23	Cis-9,12-Octadecanoic acid
γ- Linolenic acid *	34.21	24.79	Cis-9,12,15-Octadecanoic acid
Brassidic acid *	36.28	0.06	Trans-13- Docosenoic acid
Arachidic acid	37.40	1.4	Eicosanoic acid
U	38.38	1.73	
Erucic acid *	40.46	2.06	Cis-13-docosenoic acid
Nervonic acid*	41.08	1.69	Cis-15-Tetracosenoic acid
Unsaturated fatty acids		83.31 % of total fatty acids	
Saturated fatty acids		13.26 % of total fatty acids	

*: Unsaturated fatty acid.
U: undetected fatty acid methyl ester.

Effect of BO on Some Biomarkers of Hepatotoxicity

Data presented in Table (2) showed that exposure of rats to 6.5 Gy of whole body γ-radiation induced a significant increase ($p<0.001$) in the activities of AST, ALT and GGT in irradiated rats compared to the corresponding levels in non-irradiated control rats at the 7^{th} and 15^{th} days post radiation exposure. While both irradiated BO post and prepost-treated groups showed significant amelioration in the serum levels of ALT, AST and GGT when compared to the corresponding irradiated group, there were significant decrease ($p<0.001$) at the 15^{th} day when compared with irradiated group. It was found that, BO prepost-treatment had more powerful effect against irradiation induced hepatotoxicity than BO post-treatment.

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Table 2: Effect of BO on serum transaminases (AST&ALT) and gamma glutamyl transferase (GGT) levels in irradiated (Irrad) rats

Parameters	Time intervals (Day)	Experimental groups				
		Control	Irrad	BO	Irrad BO post-treated	Irrad BO prepost-treated
AST (U/L)	7th	108.98±9.85	a*** 138.12±12.65	b*** 106.22±9.28	a** b** c** 122.59±9.49	a* b** c** 120.92±7.11
	15th	106.97±9.64	a*** 136.45±11.40	b*** 104.56±8.50	a* b***c* 117.93±10.58	b*** 115.09±9.49
ALT (U/L)	7th	41.04 ± 2.59	a*** 54.56 ± 5.35	b*** 41.03±4.01	a** b*c** 49.76 ± 4.69	a* b** 46.61 ± 3.64
	15th	42.03 ± 4.21	a*** 53.72±4.89	b*** 41.20±4.15	a* b**c* 46.94±4.06	b*** 45.77± 3.73
GGT (U/L)	7th	4.43±0.38	a*** 9.24±1.33	b*** 4.7±0.76	a***b***c* 6.45±0.99	b*** 4.58±0.77
	15th	4.33±0.33	a*** 8.74±0.9	b*** 5.00±0.52	b*** 5.19±0.82	b*** 4.58±0.77

Values were represented as means ± SD (n=6).
Statistical analysis was done between groups in the same time interval.
[a] Significant difference between control and irradiated groups. [b] Significant difference between irradiated and irradiated BO post and prepost-treated groups. [c] Significant difference between non-irradiated BO and irradiated BO post and prepost-treated groups. [d] Significant difference between irradiated BO post-treated and irradiated BO prepost-treated groups. (*p<0.05, **p< 0.01 and *** p< 0.001)

Effect of BO on Reduced Glutathione (GSH) and Lipid Peroxide (MDA) in Irradiated Rats

In irradiated group, serum and hepatic GSH activities were significantly reduced at both the 7th and 15th days post irradiation as compared with their corresponding levels in control group. However, irradiated BO post-treated and prepost-treated groups showed significant increase in GSH activities during the experimental periods (7th and 15th days) as compared with irradiated group. The hepatic and serum levels of MDA in irradiated rats showed significant increase at the 7th and 15th days compared with their corresponding levels in control group. However, both irradiated BO post-treated and prepost-treated groups induced significant improvement in the serum and hepatic MDA when compared with their levels in irradiated group at the 7th and 15th days post irradiation Tables (3&4).

Effect of BO on Lipids Profile of Irradiated Rats

Table (5) showed the effect of BO on the serum levels of lipids profile of irradiated rats. In irradiated group the levels of TG, TC, and LDL-C revealed a significant increase (p<0.001), while HDL-C level recorded a significant decrease (p<0.001) at the 7th and 15th days post irradiation as compared to the corresponding values in control rats. Lipids profile parameters were significantly improved in both irradiated BO post-treated and prepost-treated groups as compared with irradiated group. The improvement was more pronounced in irradiated BO prepost-treated group than irradiated BO post-treated group.

Table 3: Effect of BO on serum reduced glutathione (GSH) and lipid peroxide (MDA) levels in irradiated (Irrad) rats

Parameters	Time intervals (Day)	Experimental groups				
		Control	Irrad	BO	Irrad BO post-treated	Irrad BO prepost-treated
GSH (mg/dl)	7th	68.34 ± 6.59	a*** 32.33±6.33	b*** 70.13±5.47	a**b***c* 54.45±4.15	a*b***c* 57.95±10.38
	15th	68.02 ± 6.94	a*** 36.23±5.90	b*** 69.06±5.63	b*** 60.22±6.73	b*** 63.32±5.86
MDA (nmol/ml)	7th	73.04 ± 7.01	a*** 135.22 ± 9.6	b*** 69.54±6.21	a**b***c* 88.14±8.27	b*** 70.64±6.17
	15th	72.53 ± 6.69	a*** 126.32 ±7.18	b*** 70.82 ±6.15	b***c* 79.52±7.83	b*** 69.48±5.47

Values were represented as means ± SD (n=6).
Statistical analysis was done between groups in the same time interval. [a] Significant difference between control and irradiated groups. [b] Significant difference between irradiated and irradiated BO post and prepost-treated groups. [c] Significant difference between non-irradiated BO and irradiated BO post and prepost-treated groups. [d] Significant difference between irradiated BO post-treated and irradiated BO prepost-treated groups. (*p<0.05, **p< 0.01 and *** p< 0.001)

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Table 4: Effect of BO on hepatic reduced glutathione (GSH) and lipid peroxide (MDA) levels in irradiated (Irrad) rats

Parameters	Time intervals (Day)	Experimental groups				
		Control	Irrad	BO	Irrad BO post-treated	Irrad BO prepost-treated
GSH (mg/g tissue)	7th	49.97±4.65	31.15 ± 3.03 a***	51.99 ± 4.75 b***	42.88±4.49 a**b***c**	43.72±4.49 a*b***c**
	15th	49.64 ±4.31	32.65 ± 2.82 a***	50.33 ± 4.88 b***	44.05±4.42 a*b***c*	45.38±4.51 b***
MDA (nmol/g tissue)	7th	163.24 ± 9.49	192.22±11.65 a***	161.18±6.90 b***	175.43±6.74 a*b**c**	174.10 ±9.89 a*b***c*
	15th	164.35 ± 8.16	188.56 ±10.28 a***	159.34±6.11 b***	171.10± 9.12 b***c*	168.43±6.24 b***

Values were represented as means ± SD (n=6).
Statistical analysis was done between groups in the same time interval. [a] Significant difference between control and irradiated groups. [b] Significant difference between irradiated and irradiated BO post and prepost-treated groups. [c] Significant difference between non-irradiated BO and irradiated BO post and prepost-treated groups. [d] Significant difference between irradiated BO post-treated and irradiated BO prepost-treated groups. (*p<0.05, **p< 0.01 and *** p< 0.001)

Table 5: Effect of BO on serum lipids profile parameters in irradiated (Irrad) rats

Parameters	Time intervals (Day)	Experimental Groups				
		Control	Irrad	BO	Irrad BO post-treated	Irrad BO prepost-treated
TG (mg/dl)	7th	91.79±6.92	146.85±15.33 a***	89.41±7.21 b***	104.04±7.75 a*b***c*	100.88±5.97 b***c*
	15th	93.80±7.83	136.77±10.04 a***	90.41±8.43 b***	100.99±8.3 b***	97.94±8.51 b***
TC (mg/dl)	7th	87.12±4.29	151.09±10.39 a***	88.46±4.47 b***	104.72±11.83 a***b***c*	98.38±7.95 a**b***c*
	15th	89.72±6.90	140.09±8.24 a***	89.45±6.51 b***	98.13±5.68 b***	95.32±6.11 b***
HDL-C (mg/dl)	7th	42.34±6.56	24.12± 4.5 a***	45.48±3.87 b***	39.22±3.27 b***c*	40.72±3.68 b***
	15th	43.27±6.69	30.05±4.36 a***	44.43±3.71 b***	41.14±2.61 b***	43.69±6.07 b***
LDL-C (mg/dl)	7th	25.50± 3.68	97.51±7.11 a***	25.51±6.50 b***	44.54±9.37 a***b***c*	37.21±6.35 a**b***c* d*
	15th	27.76±6.46	82.51±8.23 a***	26.51±6.77 b***	35.55±7.62 b***c*	32.45±7.07 b***

Values were represented as means ± SD (n=6).
Statistical analysis was done between groups in the same time interval. [a] Significant difference between control and irradiated groups. [b] Significant difference between irradiated and irradiated BO post and prepost-treated groups. [c] Significant difference between non-irradiated BO and irradiated BO post and prepost-treated groups. [d] Significant difference between irradiated BO post-treated and irradiated BO prepost-treated groups. (*p<0.05, **p< 0.01 and *** p< 0.001)

Histopathological Examination

Microscopical examination of liver tissues from control rats showed normal histological structural of hepatic lobule (Fig 2(1)). Irradiation induced marked changes in liver tissues. Liver sections of irradiated rats showed hepatic necrosis area associated with leucocytic inflammatory cells at the 7th day (Fig 2(2-a &b)), while at the 15th day showed focal hydropic degeneration of hepatocytes and portal infiltration with mononuclear inflammatory cells (Fig 2(3-a)), with focal perivascular leucocytic cells infiltration (Fig2 (3-b)). Liver sections in rats that were given BO revealed no histological alterations (Figs 3 (1)). In the irradiated group that was post-treated with BO liver sections showed congestion of hepatoportal blood vessels with portal infiltration at the 7th day (Fig 3 (2)), while at the 15th day, revealed normal histological structure (Fig 3 (3)). In the irradiated BO prepost-treated group, liver sections showed slight dilatation of hepatic sinusoids at the 7th day (Fig 3 (4)), while at the 15th day, examined sections revealed normal histological structure (Fig 3 (5)).

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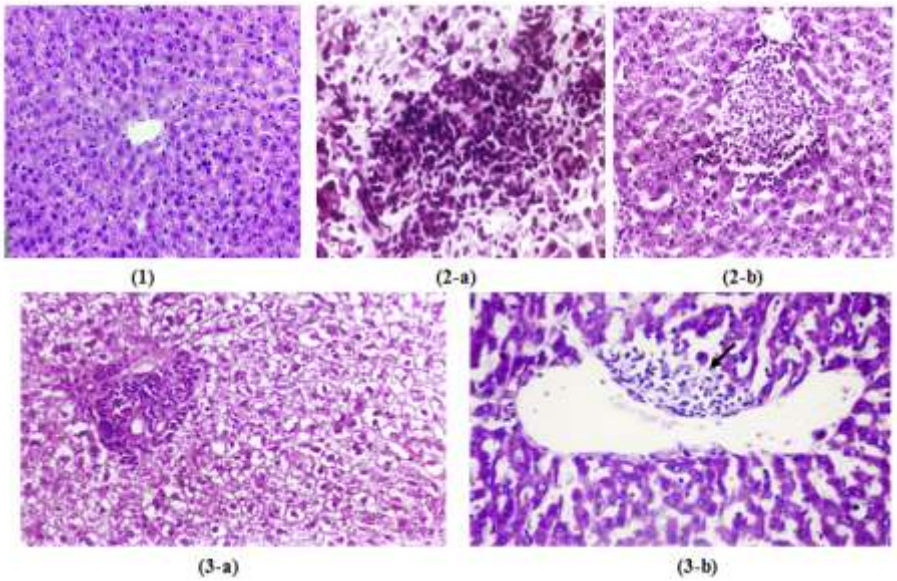

Figure 2: Liver sections from control showing normal structure (1). Liver sections of irradiated rats at the 7th day showing hepatic necrosis area associated and replaced with leucocytic inflammatory cells (2-a &b). while at the 15th day showing focal hydropic degeneration of hepatocytes and portal infiltration with mononuclear inflammatory cells (3-a) (H & E x 200), with focal perivascular leucocytic cells infiltration (arrow) (3-b) (H & E x 400).

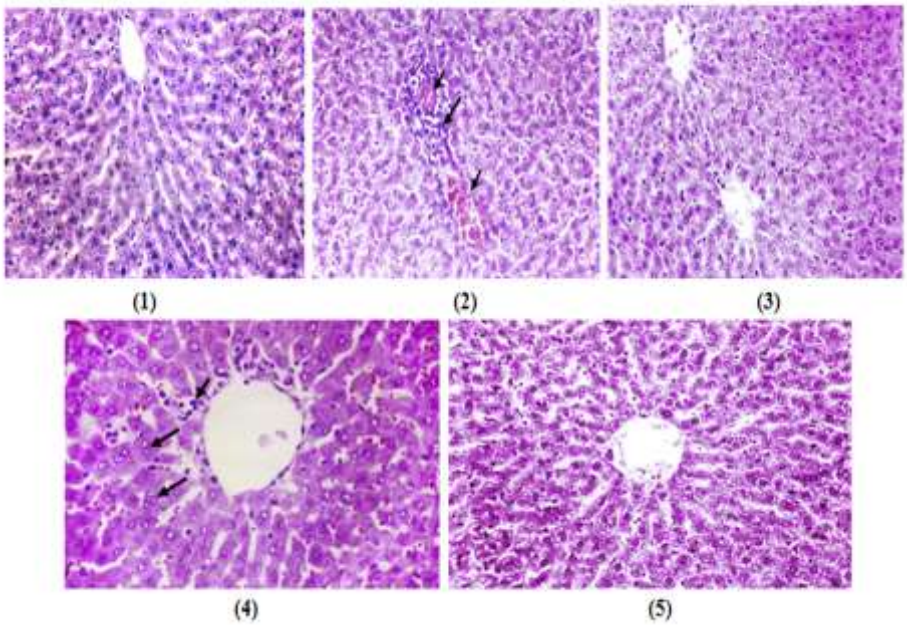

Figure 3: Liver from BO group showing no histopathological alterations (1). Liver sections of irradiated BO post-treated rats at the 7th day showing hepatoportal blood vessels congestion (small arrows), as well as portal infiltration with mononuclear cells (large arrow) (2), while at the 15th day showing apparent normal structure (3). Sections of irradiated BO prepost- treated rats at the 7th day showing slight dilatation of hepatic sinusoids (small arrow) with few leucocytes in hepatic sinusoids and binucleation of hepayocytes (large arrows) (4) (H&E x 400), while at the 15th day showing normal histological structure (5) (H & E x 200).

Discussion

Reactive oxygen species (ROS) induce many changes in physiological and pathological states (Tawfik et al., 2006). They may be toxic through attacking the molecular level directly or indirectly by generating secondary reactive species (Brenneisen et al., 1997). These radicals may cause biomolecules oxidative damage (Kowaltowski and Vercesi,

1999). Borage oil is of great interest due to its high content of GLA (Bandoniene and Murkovic, 2002). This essential polyunsaturated fatty acid plays a role in health maintenance through involvement in cell membrane structure and synthesis of anti-inflammatory eicosanoids such as prostaglandins E_1 which have antioxidant and hepatoprotective properties (Lukivskaya et al., 2006). Borage seeds oil of the present study contained high percentage concentrations of linoleic (34.23%) and γ-linolenic (24.79%) acids. These fatty acids profile and concentrations are near to that reported in previous studies by Eskin (2008) and Tasset-Cuevas et al. (2013). Total unsaturated fatty acids (83.31%) were higher than total unsaturated fatty acids (57.67%) observed by El-Gengaihi et al. (2004) but similar to that observed by Khan and Shahidi (2000).

Liver enzymes and GGT are reliable markers of hepatotoxicity. The obtained data revealed significant increase in AST, ALT and GGT activities in irradiated rats throughout the experimental period. The observed increase in the hepatotoxicity biomarkers agree with the previous studies (Ashry and Hussein, 2007; Mansour and El-Kabany, 2009). The increase in the level of liver enzymes might be a result of the damage in hepatic cellular membranes (Gaur and Bhatia, 2009), impair of intrahepatic and extrahepatic bile flow, injury of hepatobiliary, or destruction of erythrocyte induced by irradiation (Singh et al., 2011). In this study, BO ameliorated γ- irradiation-induced hepatic injuries in rats. Our results agree with the previous findings showing that BO has hepatoprotective effect by reducing the production of pro-inflammatory mediators (Engler and Engler, 1998; Chen-Yang et al., 2014). Borage oil hepatoprotection is connected with its antioxidant properties (Lukivskaya et al., 2012). The phytochemical constituents, tocopherols and GLA are responsible for this hepatoprotective effect of borage seeds oil (Eskin, 2008; Soto et al., 2008).

The present results revealed significant acceleration in serum and hepatic MDA levels associated with depletion in serum and hepatic GSH levels due to radiation exposure. These results agree with, El-Dawy et al. (2007) and Mansour et al. (2014a) who reported that irradiation induced marked oxidative stress as evidenced by the significant increase in MDA with the significant decrease in GSH levels. The increase in MDA levels might be due to the interaction of free radicals with polyunsaturated fatty acids in the phospholipids portion of cellular membranes (Prasad et al., 2005). The decrease in GSH levels might be due to its consumption during the oxidative stress induced by ionizing radiation (Mansour, 2013). However, BO induced significant improvement in serum and hepatic antioxidant status. Previous studies indicated that borage seeds oil was one of the richest plant that contains γ-linolenic acid (Kotnik et al., 2006; Asadi-Samani et al., 2014). The high levels of GLA in BO might have accentuated the treating action of this oil, where γ-linolenic acid is needed for the synthesis of hormone-like prostaglandins, which regulate certain aspects of metabolism, protect against prooxidants induced membrane and cellular damage, and possess a modulatory action on the antioxidant status. Several studies evaluated the relationship between antioxidant activity of borage extract and its GLA content (De Haro et al., 2002; Del Río-Celestino et al., 2008). Tasset-Cuevas et al. (2013) have shown that both borage seed oil and GLA were able to desmutagenise the genotoxic activity of hydrogen peroxide by scavenging the ROS originated by the model genotoxicant used. The protective effect of BO on liver in this study can be related to its function of scavenging free radicals and to its high content of GLA.

Concerning lipids profile, the present study showed that irradiation induced hyperlipidemia in the form of significant increase in serum levels of TG, TC and LDL-C and decreased levels of HDL-C compared to the control group. These data are in agreement with earlier studies by Mansour (2013) and Mansour et al. (2014b). Several mechanisms have been postulated to explain such effects. The stimulation by gamma irradiation of liver enzymes responsible for the biosynthesis of fatty acids and mobilization of fat from adipose tissues to the blood stream leads to hyperlipidemic state (Darwish et al., 2007). Also, hormonal imbalance, in which the high insulin or the low glucagon level enhances the synthesis of triacylglycerol in both adipose tissues and liver, which is accompanied by the acceleration of fatty acids mobilization from the fat deposits to the blood (Baker et al., 2009). The amelioration of lipids parameters by BO may be attributed to γ-linolenic acid (prostaglandin precursor), which has been known to improve insulin-mediated glucose metabolism and abnormal lipids profile (Certik, 1993). The obtained results are in accordance with El-Gengaihi (2004) who found that administration of borage oil to rats led to a significant improvement in lipids parameters. In addition, Seman et al. (1999) found that GLA, as a content of borage oil, has great hypocholesterolemic ability in humans.

In the present study, irradiation produced damage reflected by deleterious changes in the structure of liver tissues. These results agree with Chen et al. (2001) and Soliman et al. (2007) who found that gamma irradiation induced injury, swelling, and pathological damage of rats' liver. The pretreatment with antioxidant agent prevents the injurious effect of irradiation on rats' liver. These findings may be due to the fact that exposure to radiation causes injury to blood vessels with degeneration and necrosis of hepatic parenchyma (Jirle et al., 1990). However, BO has a protective effect against morphological changes induced by γ-irradiation, and the improvement is more pronounced in both irradiated BO post-treated and prepost-treated groups at the 15th day. This may be due to the fatty acids composition of BO, which promises the useful protector against the production of free radicals induced by gamma irradiation (Kawashima et al., 2002).

Conclusion

In conclusion, oral administration of BO, which is rich in γ-linolenic acid (GLA), significantly lower γ-irradiation-induced oxidative damage and hepatotoxicity in rats. The mechanisms of BO that provide protection against γ-

irradiation-induced toxicity may be explained by its antioxidant activity, inhibition of MDA, and prevention against GSH depletion due to its high content of GLA. Therefore, BO may be used as a beneficial supplement for patients during radiotherapy treatment. and

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