

Original Article

POTENTIAL ROLE OF MILK THISTLE SEED AND ITS OIL EXTRACTS AGAINST HEART AND BRAIN INJURIES INDUCED BY γ -RADIATION EXPOSURE

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ABSTRACT

Objective: This study aimed to investigate the protective effect of *Silybum marianum* (*S. marianum*) seeds extract its oil fraction against damage effect of γ -radiation in female albino rats.

Methods: Ultrasonic-assisted extraction was used for the extraction of *S. marianum* seeds. Lipid patterns of *S. marianum* seeds oil were elucidated using gas chromatography-mass spectrometry (GC-MS). *S. marianum* seeds extract was analyzed using high-performance liquid chromatography (HPLC). Malondialdehyde (MDA), reduced glutathione (GSH) and metallothionein (MT) were estimated in heart and brain tissues of the examined rats. Lactate dehydrogenase (LDH) and creatine kinase-MB (CKMB) were measured in the serum of the examined rats, and the brain biomarkers; dopamine and serotonin were also measured.

Results: The oil was found to be rich in linoleic acid (58.20%) and arachidic acid (23.38%). *S. marianum* seeds extract revealed the presence of taxifolin and six main active constituents of *silymarin*, including silydianin, silychristin, silybin A, silybin B, isosilybin A and isosilybin B. Treatment of γ -radiation damage effect using *S. marianum* seeds extract and its oil fraction led to a significant reduction of MDA levels in heart (139.6 and 165.5 nmol/g, respectively) and brain (158.5 and 135.2 nmol/g, respectively) tissues, however, significant increase of GSH levels in heart (316.4 and 293 mg/g, respectively) and brain (210.4 and 227 mg/g, respectively) tissues was observed, also a significant increase of dopamine levels (85.27 and 65.74 ng/g, respectively) and MT levels of heart tissues (108.5 and 70.52 mg/g, respectively) was observed.

Conclusion: *S. marianum* seeds extract and its oil fraction showed a protective effect against γ -radiation-induced damage in heart and brain.

Keywords: *Silybum marianum*, γ -radiation, Heart, Brain, Female rats

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INTRODUCTION

Reactive oxygen species (ROS) are a normal component of oxidative phosphorylation and play an important role in normal redox control of physiological signalling pathways. However, excessive ROS generation triggers cell dysfunction, lipid peroxidation, DNA mutagenesis and can lead to irreversible cell damage or death [1-3]. Reactive oxygen compounds may attack and damage lipids, proteins and DNA. Such effects appear to contribute in the pathogenesis of many diseases, such as central nervous system diseases, cancer, cardiovascular diseases and liver damage. Antioxidants, as flavonoids, have positive effects on preventing or attenuating these diseases [4].

Radiation exposure is an oxidative stress inducer via generation of ROS such as superoxide anions, hydrogen peroxide and hydroxyl radicals [5]. Therefore, protection of the biological systems from ionizing radiation is of paramount importance to combat radiation damages using nontoxic radio protectors. Many synthetic, as well as natural compounds, have been investigated for their efficacy to protect the biological systems against the deleterious effects of radiations [6, 7].

Silybum marianum L. Gaertn (*S. marianum*) seeds (milk thistle, *Asteraceae*), contain an isomeric mixture of polyphenolic flavonolignans (silychristin, silydianin, silybin, and isosilybin), collectively known as *silymarin* [8]. *Silymarin* has a long history of use, it has antioxidant, anti-inflammatory and antifibrotic effects in the liver [9, 10], and it may be used as an anticancer agent [11]. *Silymarin* or silybin alone showed hepatoprotection effect against iron overload [12] and chemical toxicity [13]. Also, it can enhance regeneration and metabolism of liver cells [14, 15], including bile salt production [16]. According to Prabha and co-workers [17], silibinin showed a protection effect against radiation-

induced mortality and DNA damage in blood leukocytes. Also, silybin and its analogues offer different ability to repair DNA base against radiation-induced damage [18]. *Silymarin* may reduce oxidative stress in the brain, with potential consequences for neurodegenerative disorders and may contribute to the prevention of age-related and pathological degenerative processes in the brain [19].

The present study aimed to investigate the protective effect of *S. marianum* seeds extract or/and its oil fraction against the exposure to fractionated dose of γ -radiations.

MATERIALS AND METHODS

Plant material

S. marianum seeds were collected from the field, Beni-suif, Egypt (GPS coordinates: N29 °16.518', E031 °16.561', elevation 63 m), after cultivation for one year during (September 2014) and a voucher specimen (#6411) was deposited at the National Research Centre Herbarium. Collected seeds were washed with distilled water and dried, then grinded to powder and stored for further extraction.

Chemicals

Silymarin standard (Sigma-Aldrich Company Ltd.) was purchased from VWR International (East Grinstead, West Sussex, UK).

Experimental animals

Female albino Wistar rats weighing 150±20 g were obtained from the Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt. Animals were kept in plastic cages and under good ventilation and illumination conditions and allowed free access to tap water and food.

All experimental protocols were approved by "Medical Research Ethics Committee", National Research Centre, Al Buhouth St., Dokki-Cairo, Egypt, No. 17-043

Preparation of *Silybum marianum* seeds oil and *Silybum marianum* seeds extract (Silymarin)

Extraction of *Silybum marianum* seeds oil

About 500 g of finely powdered seeds were extracted using *n*-hexane for 15 min. Ultrasonic probe with a tip diameter of 20 mm was employed for direct sonication (the horn tip position inside the extraction vessel was 1 cm under the solvent level). Extraction was carried out using an Ultrasonic Processor UP400S (400 watts, 24 kHz, Hielscher). Extraction was carried for (15 min) at room temperature (temperature was monitored by a thermocouple inside extraction mixture by using an ice cooling bath around the extraction vessel to keep the temperature constant at 25 °C±5 °C). After extraction, the extract was centrifuged at 4000 rpm.

The supernatant was evaporated under vacuum at 40 °C to a constant weight and yellow colored residual oil was dried over anhydrous sodium sulfate.

Preparation of unsaponifiable matter

S. marianum seeds oil (1.45 g) was dissolved in boiling acetone (100 ml), cooled and the amorphous formed precipitate was separated out. The acetone soluble fraction was saponified (N/2 alc. KOH) and the unsaponifiable matter (0.30 g) was separated [20].

Preparation of saponifiable matter (fatty acids)

The aqueous alkaline solution left after the separation of the unsaponifiable matters was acidified with 5% Sulphuric acid (H₂SO₄) and the liberated fatty acids were extracted with diethyl ether till exhaustion. The ether extracts containing the free fatty acids were combined, washed with distilled water (2 × 50 ml) till free from acidity and dehydrated over anhydrous sodium sulphate and then filtered [20]. The solvent was evaporated to dryness under reduced pressure. The weight of the residue was 0.56 g.

Preparation of fatty acid methyl esters

0.56 g of the fatty acid fraction was methylated by dissolving in 10 ml methanol and 5 ml of boron trifluoride (BF₃). The mixture was refluxed for 5 min on a boiling water bath. The mixture was cooled and the methanol was evaporated under reduced pressure. The residue was diluted with 20 ml distilled water and then extracted with diethyl ether (5 × 30 ml). The combined ethereal extracts were washed with distilled water till neutral to litmus paper, dried over anhydrous sodium sulphate, filtered and evaporated under reduced pressure till dryness [20]. The weight of the residue was 0.51 g representing 51% of the total lipoidal matter. The fatty acids methyl esters were subjected to gas chromatography-mass spectrometry (GC-MS) analysis.

Gas chromatography-mass spectrometry analysis of the unsaponifiable matter

GC-MS analysis of the unsaponifiable matter was carried out using gas chromatography-mass spectrometry, TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system equipped with a TG-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The injected volume was 0.2 µl of diluted sample (1:10 hexane, v/v). Analysis was carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 50 °C for 3 min; rising at 5.0 °C/min to 300 °C and held for 20 min. The injector and detector were held at 280 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450.

Gas chromatography-mass spectrometry analysis of the fatty acids methyl esters

GC-MS analysis of the fatty acids methyl esters was carried out on the same device used for the unsaponifiable matter with the same injection volume, flow rate, split ratio, and carrier gas. However,

temperature program was as follow: 80 °C for 1 min; rising at 4.0 °C/min to 300 °C and held for 1 min. The injector and detector were held at 240 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450.

Silymarin extraction

The marc left after defatting was then thoroughly extracted using ethyl acetate, extraction was carried out using an ultrasonic probe with a tip diameter of 20 mm (the horn tip position inside the extraction vessel was 1 cm under the solvent level). Extraction was carried out using an Ultrasonic Processor UP 400S (400 watts, 24 kHz, Hielscher), direct sonication for (15 min) at room temperature (temperature monitored by a thermocouple inside extraction mixture by using an ice cooling bath around the extraction vessel to keep the temperature constant at 25 °C±5 °C) [21]. After extraction, the collected extract was filtered throughout a Fisher brand QL100, 150 mm filter paper, then the supernatant was evaporated till dryness under reduced pressure at 45 °C, weighed and stored at -18 °C for high-performance liquid chromatography (HPLC) analysis.

Preparation of standards

A methanolic solution of standard *silymarin* (0.7 mg/ml) was used to understand the chromatographic behaviour of the flavonolignan components of *silymarin* in analytical conditions.

High-performance liquid chromatography device specifications

HPLC (Agilent 1100 series) was used to determine the chemical composition of each extract as well as the standard *silymarin*, equipped with G1315 B diode array detector (DAD), G1313A Autosampler, G1311A Quaternary Pump, G1322A Vacuum Degasser, G1321A Fluorescent Detector, G1316A Column Comp. The control system and data acquiring system was installed with Agilent Chemo station for LC system.

High-performance liquid chromatography analysis

S. marianum seeds extract and standard *silymarin* was injected separately to Semi-prep HPLC for analysis using different proportional of H₂O and methanol as mobile phase.

The mobile phase used was 90:10:1 methanol: H₂O: formic acid [solvent A] and H₂O (containing 0.1% formic acid) [solvent B] at a flow rate of 0.5 ml/min, the oven was set at ~25 °C. Injection volume was 5 µl. Detection was carried out by monitoring the absorbance signals at 288 nm.

Radiation processing

The whole body of the examined rats was exposed to γ -irradiation using ¹³⁷Cs, biological irradiator source (γ -Cell-40), located at the National Centre for Radiation Research and Technology (NCRRT), Egypt. The cesium source provides a dose rate of 0.43Gy/min at the time of the experiment. The whole body of animals was exposed to fractionated dose of gamma-rays (4 GyX 2).

Experimental design

After adaptation period for two weeks, animals were divided to six groups, each of eight rats. Group 1 (G1): control, non-irradiated rats. Group 2 (G2): the whole body of rats was exposed to fractionated dose of γ -radiation 4 Gy X 2 (four days interval). Group 3 (G3): rats orally received *S. marianum* seeds extract, 100 mg/kg body weight (b. wt.) [22] daily for 12 consecutive days. Group 4 (G4): rats orally received *S. marianum* seeds extract, 100 mg/kg b. wt. daily for 12 consecutive days and the whole body of rats was exposed to fractionated dose of γ -radiation 4 Gy X 2 (at 4th and 8th day of treatment). Group 5 (G5): rats orally received *S. marianum* seeds oil fraction, 0.5 ml/kg b. wt. daily for 12 consecutive days. Group 6 (G6): rats orally received *S. marianum* seeds oil fraction, 0.5 ml/kg b. wt. daily for 12 consecutive days and the whole body of rats was exposed to fractionated dose of γ -radiation 4 Gy X 2 (at 4th and 8th day of treatment). Rats were sacrificed at 13th day of treatment or after 4 d from the second exposure of γ -irradiation.

Biochemical analysis

Blood samples were collected by cardiac puncture under diethyl ether anesthesia, and then serum was obtained by centrifugation at

3000 rpm for 15 min. Heart and brain were quickly excised from the rats, washed in ice-cold saline and homogenized (1:5 w/v) in 0.25M sucrose. Lactate dehydrogenase (LDH) serum levels were measured as reported [23], using UV/VIS Spectrophotometer. Creatine kinase-MB (CKMB) serum levels were estimated as reported [24], using commercial kit of ELITECH. Heart and brain reduced glutathione (GSH) levels were estimated according to the method described by Beutler and co-workers [25]. Malondialdehyde (MDA) was estimated as TBARS using UV/VIS Spectrophotometer [26]. Metallothionein (MT) levels were determined by Ag-saturation hemolysate method as reported [27, 28], using Thermo Scientific iCE 3000 SERIES Atomic Absorption Spectrometry. A portion of the brain was prepared for dopamine and serotonin estimation as reported [29], using Spectrophotofluoromete.

Statistical analysis

Student's t-test was applied for the statistical analysis of collected data to determine the probable level of significance. The differences were considered significant at $P \leq 0.05$ [30].

RESULTS

Fatty acids pattern

The fatty acid composition of cultivated *S. marianum* seeds oil is recorded in table 1. Results revealed the presence of a mixture of 10 fatty acids, and it was found that *S. marianum* seeds oil is rich in unsaturated fatty acids, which represent 76 % of the total fatty acids. Linoleic acid and arachidic acid are the major constituents and form 58.20 % and 23.38 % of the total composition, respectively.

Table 1: Fatty acid composition of *Silybum marianum* seeds oil fraction

Peak No.	RRT	Relative %	Constituents
1	0.896	0.25	C ₁₆₍₀₎ Palmitic acid
2	0.980	0.01	C ₁₆₍₁₎ Palmitoleic acid
3	0.983	0.03	C ₁₇₍₀₎ Heptadecanoic acid
4	0.985	0.04	C ₁₇₍₁₎ Heptadecenoic acid
5	0.987	0.03	C ₁₈₍₀₎ Stearic acid
6	0.990	17.87	C ₁₈₍₁₎ Oleic acid
7	1	58.20	C ₁₈₍₂₎ Linoleic acid
8	1.001	23.38	C ₂₀₍₀₎ Arachidic acid
9	1.006	0.17	C ₂₀₍₁₎ Eicosenic acid
10	1.090	0.01	C ₂₂₍₀₎ Behenic acid

Sterols pattern

Sterols pattern of the complete unsaponifiable fraction is shown in table 2. The sterol fraction of the *S. marianum* seeds oil fraction

consisted mainly from a mixture of sterols (cholesterol, β -sitosterol, stigmasterol, campesterol and γ -sitosterol) and one triterpene β -amyryn (6%). γ -sitosterol is the major component of *S. marianum* sterol and forms (45.81%) of the total composition.

Table 2: Sterol composition of cultivated *Silybum marianum* seeds oil fraction

Peak No.	RRT	Relative %	Constituents
1	0.952	15.45	Cholesterol
2	0.978	4.88	Campesterol
3	0.984	4.92	Stigmasterol
4	1	45.81	γ -Sitosterol
5	1.008	6.03	β -Amyryn
6	1.013	16.66	β -Sitosterol

Silymarin components

HPLC profiles of standard *silymarin* and defatted *S. marianum* seeds extract are presented in fig. 1 and fig. 2; respectively. HPLC analysis showed the presence of seven main active constituents

including taxifolin, silydianin, silychristin, diastereomers of silybin (silybin A and B) and diastereomers of isosilybin (isosilybin A and B). Seven principal peaks were observed, with each peak identified as one of the flavolignan constituents of *silymarin* (table 3).

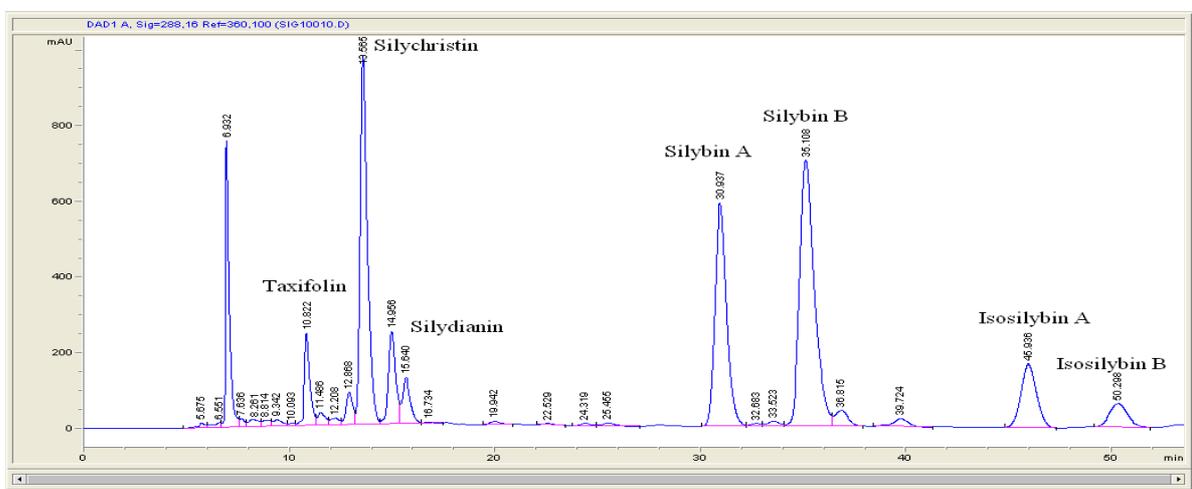


Fig. 1: HPLC chromatogram of standard *silymarin*

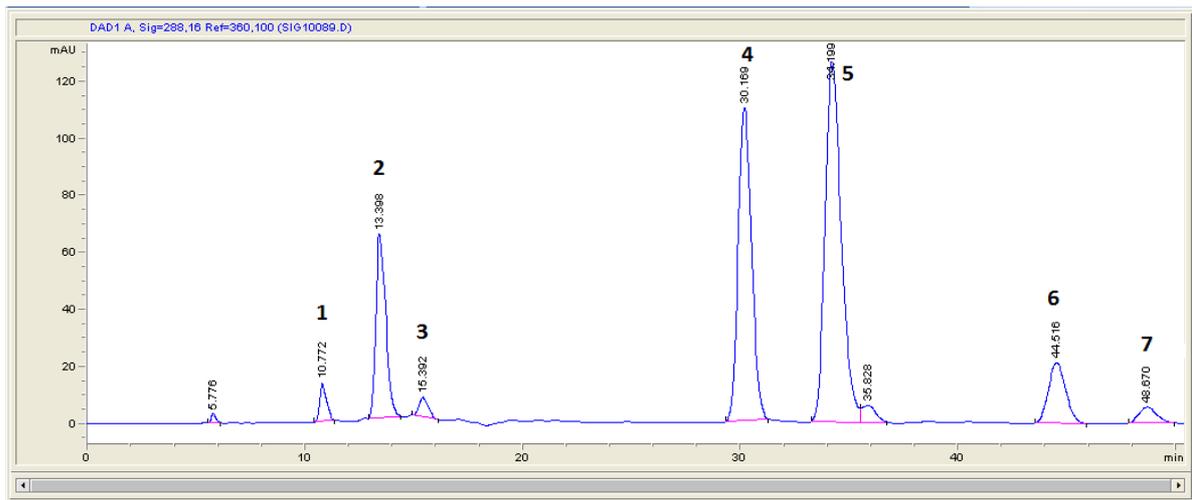


Fig. 2: HPLC chromatogram of cultivated sample of *Silybum marianum* seeds extract

Table 3: *Silymarin* composition of *silymarin* standard and cultivated seeds extract

Peak No.	Compound name	Retention time (min)	
		<i>Silymarin</i> standard	Sample
1	Taxifolin	10.822	10.772
2	Silychristin	13.565	13.398
3	Silydianin	15.64	15.392
4	Silybin A	30.937	30.169
5	Silybin B	35.108	34.199
6	Iso-Silybin A	45.936	44.516
7	Iso-Silybin B	50.298	48.67

Biochemical study

Compared to non-irradiated control group (Group 1)

Fractionated dose of γ -radiation (4 Gy X 2, four days interval) significantly elevated lipid peroxidation indicator in heart and brain tissues, MDA (table 4), however, no significant effect was observed

on GSH levels of heart and brain tissues (table 5) or MT levels of heart tissues. A significant increase of MT levels in brain tissues was observed (table 6). Irradiated rats also showed a significant decrease of LDH and CKMB serum levels (table 7). Also, a significant decrease in dopamine and serotonin levels of the brain tissues was observed (table 8).

Table 4: Effect of *Silybum marianum* seeds extract (100 mg/kg b. wt.) and *Silybum marianum* seeds oil fraction (0.5 ml/kg b. wt.) on heart and brain MDA levels

Biomarker Organs	MDA (nmol/g)	
	Heart	Brain
G1	163.2 \pm 3.760	165.2 \pm 5.273
G2	202.2 \pm 3.250*	186.2 \pm 3.212*
G3	148.1 \pm 11.86	176.2 \pm 8.601
G4	139.6 \pm 8.676*#	158.5 \pm 5.038#
G5	161.9 \pm 9.578	177.7 \pm 7.194
G6	165.5 \pm 2.548#	135.2 \pm 5.082*#

• Each value represents the mean \pm SE, n=8, *Significant differences from control at $p \leq 0.05$, #Significant differences from irradiated group at $p \leq 0.05$.

Table 5: Effect of *Silybum marianum* seeds extract (100 mg/kg b. wt.) and *Silybum marianum* seeds oil fraction (0.5 ml/kg b. wt.) on heart and brain GSH levels

Biomarker Organs	GSH (mg/g)	
	Heart	Brain
G1	187.7 \pm 8.644	172.9 \pm 9.237
G2	186.2 \pm 16.06	183.2 \pm 5.830
G3	226.0 \pm 14.07*	213.9 \pm 6.472*
G4	316.4 \pm 12.46*#	210.4 \pm 7.518*#
G5	208.4 \pm 8.614	147.9 \pm 1.780*
G6	293.0 \pm 19.50*#	227.0 \pm 4.249*#

• Each value represents the mean \pm SE, n=8, *Significant differences from control at $p \leq 0.05$, #Significant differences from irradiated group at $p \leq 0.05$.

Table 6: Effect of *Silybum marianum* seeds extract (100 mg/kg b. wt.) and *Silybum marianum* seeds oil fraction (0.5 ml/kg b. wt.) on heart and brain MT levels

Biomarker Organs	MT (mg/g)	
	Heart	Brain
G1	43.87±2.349	38.67±1.626
G2	49.95±2.698	60.44±1.813*
G3	62.70±2.700*	77.01±2.813*
G4	108.5±5.962*#	97.38±6.003*#
G5	36.10±2.154*	31.92±1.920*
G6	70.52±3.547*#	65.56±3.467*

• Each value represents the mean±SE, n=8, *Significant differences from control at p≤0.05, #Significant differences from irradiated group at p≤0.05.

G3 showed a significant increase of GSH and MT levels in both heart and brain tissues, however, no effect was observed on dopamine and serotonin levels in brain tissues or serum levels of LDH and CKMB. Also, no effect was observed on lipid peroxidation indicator in both heart and brain tissues.

G5 showed no effect on MDA levels in heart and brain tissues, but a slight decrease of GSH levels in brain and MT levels in brain and heart was observed. Also, a significant decrease of LDH, dopamine and serotonin levels was observed.

Also compared to non-irradiated control group (G1), G4 showed a significant decrease of MDA levels in heart and brain tissues, while G6 showed a significant decrease of MDA levels in brain tissues. G4 and G6 showed a significant increase of GSH and MT levels in heart

and brain tissues. On the other hand, G4 and G6 showed a reduction in serum LDH and CKMB levels. Results also showed a significant increase of dopamine levels in G4.

Compared to irradiated control group (Group 2)

G4 and G6 showed significant reduction of MDA levels and significant increase of GSH levels in both heart and brain tissues. G6 showed a significant increase of MT levels of heart tissues. G4 showed significant reduction of serum levels of LDH and CKMB, in addition, elevation in dopamine and serotonin levels of brain tissues was observed. On the other hand, G6 showed elevation in dopamine levels, however, no significant effect was observed on serum levels of LDH and CKMB or serotonin levels of brain tissues.

Table 7: Effect of *Silybum marianum* seeds extract (100 mg/kg b. wt.) and *Silybum marianum* seeds oil fraction (0.5 ml/kg b. wt.) on serum levels of LDH and CKMB

Biomarker	LDH (U/l)	CKMB (U/l)
G1	1676±102.2	154.3±11.95
G2	1163±102.4*	76.38±2.025*
G3	1971±180.1	156.2±12.71
G4	804.2±44.69*#	56.50±4.811*#
G5	1168±110.5*	179.0±12.32
G6	1390±61.62*	84.34±2.936*

•Each value represents the mean±SE, n=8, *Significant differences from control at p≤0.05, #Significant differences from irradiated group at p≤0.05.

Table 8: Effect of *Silybum marianum* seeds extract (100 mg/kg b. wt.) and *Silybum marianum* seeds oil fraction (0.5 ml/kg b. wt.) on dopamine and serotonin levels

Biomarker	Dopamine (ng/g)	Serotonin (µg/g)
G1	66.53±1.074	7.200±0.208
G2	38.80±1.804*	3.300±0.265*
G3	70.40±6.052	6.207±0.306
G4	85.27±4.321*#	7.247±0.653*#
G5	43.77±2.486*	3.740±0.151*
G6	65.74±3.282*#	4.046±0.252*

•Each value represents the mean±SE, n=8, *Significant differences from control at p≤0.05, #Significant differences from irradiated group at p≤0.05.

DISCUSSION

γ-radiation exposure increases generation of ROS in different tissues [5]. Increasing of ROS may be involved in atherosclerosis, myocardial ischemia/reperfusion injury and heart failure [31]. High doses of radiation applied to the heart during radiotherapy used in breast cancer [32, 33], Hodgkin's disease [34] or childhood cancers [35], increases cardiovascular malfunctions incidence and mortality. It has been suggested that persistent changes in oxidative metabolism as a result of ionizing radiation, ultimately leads to inflammation and cardiovascular disease [31, 36]. The elevation in MDA levels in heart and brain tissues, after γ-radiation exposure, indicates the oxidative stress due to lipid peroxidation process. Epidemiological data indicates an association between increased risks of cardiovascular disease and enhanced oxidative stress in populations exposed to ionizing radiation [37-41]. Treatment of γ-

radiation damage effect using *S. marianum* seeds extract and its oil fraction led to an elevation in GSH and MT levels in heart and brain tissues. MT has an important role as a free radical scavenger [42]. Consequently, MT can protect from γ-radiation damage. Galhardi and co-workers [19] demonstrated the protective effect of *silymarin* on the antioxidant defence systems in the brain of rats, inducing an increase of glutathione, ascorbic acid and superoxide dismutase activity, with decreased protein oxidation in hippocampus and cortex tissues. Furthermore, silybin prevented the accumulation of lipid peroxide and the decrease of the antioxidant glutathione in the hippocampus, indicating the protective effect of silybin against β-amyloid neurotoxicity, by regulating oxidative stress in the brain [43]. Also, *silymarin* was reported to induce the increase of GSH content in the cell and the inhibition of lipid peroxidation where the structure of the phenolic ring of flavonolignans showed antioxidant effect [44]. Furthermore, Fu and co-workers [45] demonstrated the

in vitro protective effect of silybin and its analogues on DNA damage, which is correlated to their ability to scavenge free radical induced by X-ray exposure.

Despite the oxidative stress induced by γ -radiation, as revealed by increased lipid peroxidation, we observed a reduction in serum levels of LDH and CKMB at γ -radiation dose of 4 Gy X 2. Meanwhile, a reduction in serum levels of LDH and CKMB of irradiated rats due to *S. marianum* seeds extract treatment was observed, compared to irradiated rats, demonstrating its radio protective effect on the heart. *S. marianum* seeds oil fraction exhibited a depletion effect on dopamine and serotonin levels whereas there was an improvement in the dopamine level in the treated irradiated rats with *S. marianum* seeds oil fraction. On the other hand, dopamine and serotonin levels gave positive results via *S. marianum* seeds extract or *S. marianum* seeds oil fraction treatment for irradiated rats. In the case of pharmacological stress caused by methamphetamine treatment, silibinin attenuated the decreases of dopamine levels in the prefrontal cortex and serotonin levels in the hippocampus [46]. *Silymarin* showed an antioxidant effect in the central nervous system, being able to cross the blood-brain barrier [47]. The antioxidant capacity of *silymarin* against peroxy radicals reduced in hippocampus and cortex of young rats and also in the hippocampus of elderly animals. This is due to the potential antioxidant effect of *silymarin* to change the redox state of the cellular environment, altering the antioxidant defence system [48]. According to Pérez and co-workers [49], 100 mg/kg b. wt. of *silymarin* showed neuroprotective effect, also at the same dose *silymarin* exhibited antidepressant effect [50]; due to acute restraint stress in mice via restoration the antioxidant enzymes.

Raza and co-workers [51] revealed that *silymarin* may be helpful in slowing down the progress of neurodegeneration in focal cerebral ischemia which suggests that neuroprotective potential of *silymarin* is mediated through its antioxidant and anti-apoptotic properties. According to Ligeret and co-workers [52], silibinin restores GSH levels, diminishes oxidative radical ($O_2^{\cdot-}$) and suppresses lipid peroxidation. Silybin and its analogues offer different ability to repair DNA base pairs against radiation-induced damage [18]. Prabha and co-workers [17] reported that oral administration of silybinin to mice resulted in significant protection against radiation-induced mortality and DNA damage in blood leukocytes.

CONCLUSION

The present study demonstrated that *S. marianum* seeds extract and its oil fraction has a protective effect against γ -radiation induced cell damage. The antioxidant activity of *S. marianum* seeds extract and its oil fraction inhibited lipid peroxidation process in heart and brain tissues and induced the production of antioxidant agents (GSH and MT) in the cells of rats exposed to γ -radiation.

S. marianum seeds extract and its oil fraction has reduced oxidative stress induced by γ -radiation exposure. Further investigation should be carried out to study the effect of *S. marianum* seeds oil fraction on brain biomarkers; dopamine and serotonin. From the present study, it could be concluded the radioprotective effect of *S. marianum* seeds extract and its oil fraction on heart and brain tissues.

AUTHORS CONTRIBUTION

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

- **Conception and design of the study:** Nohair H. Sherif, Asrar M. Hawas, Walid E. Abdallah, Ibrahim A. Saleh, Khaled A. Shams, Faiza M. Hammouda.
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- **Revising the manuscript critically for important intellectual content:** Walid E. Abdallah, Asrar M. Hawas, Ibrahim A. Saleh

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CONFLICT OF INTERESTS

Declared none

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