



## Ameliorative Effect of Silymarin Against Radiation-Induced Oxidative Stress in the Liver of Male Rats

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### ABSTRACT

Administration of dietary antioxidants has been suggested to protect against liver tissue damage induced by radiation exposure. Silymarin is a polyphenolic plant flavonoid derived from *Silymarin marianum* that has hepatoprotective and anticarcinogenic effects. Therefore, the present study was carried out to investigate the radioprotective activity of Silymarin against radiation-induced liver damage. In this work, the total phenol content of silymarin was  $0.527 \pm 0.016$  mg GAE/ mg, total antioxidant capacity was  $139 \pm 10.5$   $\mu$ mol/l and the DPPH radical scavenging activity was  $1.52 \pm 0.12$  mg/ml. The results showed that  $\gamma$ - rays (6 Gy) caused a significant increase in serum level of alanine and aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase and the level of hepatic malondialdehyde and xanthine oxidase activity. Also, a significant decrease in hepatic glutathione level and xanthine dehydrogenase, superoxide dismutase and catalase activities was observed, compared with control group. Oral administration of silymarin extract prior radiation exposure was found to offer protection against all the radiation-induced biochemical alterations. These findings showed that silymarin is a powerful antioxidant herbal drug which can protect biological systems against the oxidative stress and tissue damage induced by radiation exposure.

### Article Information

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### Authors' Contributions

HMS and ANS designed the study. HMS determined total phenol, Ferric Reducing/Antioxidant Power (FRAP) assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of silymarin. ANS provided radiation facilities. AGA collected blood samples and performed biological studies. NA contributed in biochemical analysis. All authors contributed towards preparation of manuscript.

### Key words

Silymarin; Radiation exposure; liver damage; Antioxidants.

### INTRODUCTION

Radio-therapy is one of the most common treatment modalities for human cancer and it affects not only malignant tumors but also surrounding normal tissues. Because liver is a highly radiosensitive organ (Chen *et al.*, 2015), irradiation of the non-tumor compartment of the liver may cause cell damage, changes in laboratory assessments and/or clinical signs of liver dysfunction (Cheng *et al.*, 2015). Therefore, to obtain better tumor control with higher doses of radiation, the normal tissues should be protected against radiation injury. Thus radioprotectors are of great importance in clinical radiation therapy (Maurya *et al.*, 2004).

Silymarin, a purified extract of seeds of milk thistle (*Silybum marianum* L.), is a mixture of seven major components: taxifolin, silychristin, silydianin, silybin A, silybin B, isosilybin A and isosilybin B (Anthony and Saleh, 2012). Silymarin is well known for its hepatoprotective abilities and has been evaluated for inherent utility as a radioprotective agent (Adhikari *et al.*, 2010). Silymarin has been shown to prevent damage to the liver through several mechanisms: including; inhibition of lipid peroxidation, anti-inflammation,

increase of detoxification, antioxidation and immunomodulatory effects (Momeni *et al.*, 2015).

Because silymarin can be used as a natural antioxidant agent, the aim of this work was to study the ameliorative effect of silymarin on radiation-induced liver injury in rats.

### MATERIALS AND METHODS

#### Determination of total phenol

Total polyphenols were determined by Folin-Ciocalteu (FC) procedure (Singleton *et al.*, 1999). Aliquots (0.1 ml) of extracts were transferred into the test tubes and their volumes made up to 0.5 ml with distilled water. After addition of 0.25 ml Folin-Ciocalteu reagent (FCR) and 1.25 ml 20% aqueous sodium carbonate solution, tubes were vortexed and absorbance of blue colored mixtures recorded after 120 min at 765 nm. The amount of total polyphenols was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions (covering the concentration range between 0.1 and 1.0 mg/ml), and expressed as mg gallic acid per mg dry material. All measurements were done in triplicate and values were expressed as the mean  $\pm$  SD.

#### Ferric reducing/antioxidant power (FRAP) assay

FRAP assay reported by Benzie and Strain (1996), and which depends upon the reduction of ferric [Fe(III)-TPTZ] to [Fe(II)-TPTZ] complex by a reductant at low

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pH, was adopted. This complex has an intense blue color that can be monitored at 593 nm. Assays were performed in 1.5 ml reaction mixtures containing 1.45 ml of FRAP solution and 0.05 ml of 0.1 mg/ml silymarin. Absorbance was measured at 593 nm. All experiments were done in triplicate and values were expressed as the mean $\pm$ SD.

#### *1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity*

Scavenging activity of DPPH radicals of silymarin was measured according to the method described by Brand-Williams *et al.* (1995). Assays were performed in 2 ml reaction mixtures containing 1.95 ml of 0.1 mM DPPH ethanol solution and 0.05 ml of the samples. The inhibitory effect of different concentrations of silymarin extracts (0.05-2 mg/ml) on DPPH were measured by spectrophotometric method. Absorbance of the reaction mixtures at 517nm was continuously monitored for 90 min. IC<sub>50</sub> represents the level where 50% of the radicals were scavenged by test samples.

#### *Radiation facility*

Whole body gamma irradiation of rats at a dose level of 6Gy was performed using a Canadian gamma cell-40, (137Cs) housed at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The dose rate was 0.43 Gy/min at the time of the experiment.

#### *Drug and dosage*

Pure sample of silymarin was procured from Sigma Chemicals. Silymarin was dissolved in distilled water then administered orally to the animals at therapeutic dose 50mg/kg (Naik *et al.*, 2015).

#### *Experimental animals*

Male albino rats Sprague Dawley (10 $\pm$ 2 weeks old; 120 $\pm$ 20 g) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on stock rodent diet and tap water that were allowed ad libitum. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication No. 85-23, 1996).

#### *Experimental design*

Animals (28 rats) were randomly divided into 4 groups, each of 7 animals as follows: (i) Control group rats fed on balanced diet for 8 weeks, served as control; (ii) Silymarin group rats fed on balanced diet and

received orally Silymarin (50mg/kg/day) for 8weeks; (iii) Irradiated group rats fed on balanced diet for 8 weeks and exposed to  $\gamma$ -radiation (6 Gy) at the 4<sup>th</sup> week; (iv) Irradiated and Silymarin group rats fed on balanced diet and received orally Silymarin (50mg/kg/day) for 8 weeks and exposed to  $\gamma$ -radiation at the 4<sup>th</sup> week.

At the end of the experiment, animals from each group were sacrificed 24 h post the last dose of treatment. Blood samples were collected through heart puncture after light anesthesia and allowed to coagulate and centrifuged to obtain serum for biochemical analysis. Also, liver tissue was removed for biochemical investigation.

#### *Biochemical analysis*

Liver was dissected, thoroughly washed with ice-cold 0.9% NaCl, weighed, minced and homogenized (10% w/v) using 66 mmol/L chilled phosphate buffer (pH 7.0). The homogenate that was centrifuged at 10,000 g for 20 min at 4°C was used for the estimation of malondialdehyde (MDA) and reduced glutathione (GSH). The supernatant obtained was further centrifuged at 12,000 g for 20 min at 4°C to obtain the supernatant used for the assays of superoxide dismutase (SOD), catalase activities (CAT). Lipid peroxidation was determined colorimetrically as malondialdehyde (MDA) according to Yoshioka *et al.* (1979). Hepatic xanthine oxidase (XO) and xanthine dehydrogenase (XDH) were determined according to Kaminski and Jewezska (1979). Hepatic GSH content and the activities of SOD and CAT were measured by the method of Gross *et al.* (1967), Minami and Yoshikawa (1979) and Aebi (1984), respectively. The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (1957), serum gamma glutamyl transferase (GGT) was assessed according to Rosalki (1975) and serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (1954).

#### *Statistical analysis*

Results were presented as mean $\pm$ SD (n = 7). Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. Statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS, 1998). Differences between means were considered significant at P < 0.05.

## **RESULTS**

Total phenol content of silymarin was 0.527 $\pm$ 0.016 mg gallic acid equivalent (GAE)/mg, total antioxidant capacity of silymarin (0.1 mg/ml) was 139 $\pm$ 10.5  $\mu$ mol/l

and the IC<sub>50</sub> value of silymarin on DPPH radical scavenging assay was found to be 1.52±0.12 mg/ml (Table I).

Exposure of rats to whole gamma radiation significantly increased the activity of ALT, AST, ALP and GGT as compared to normal rats. Pretreatment of irradiated rats with silymarin remarkably reduced the activities of these enzymes compared to irradiated rats (Table II).

The data presented in Table II revealed a significant increase in the level of MDA and XO activity associated with reduction in XDH activity of the liver of  $\gamma$ -irradiated rats compared to control rats. The concentration of hepatic MDA and the activity of XO were declined with an increase in the activity of XDH of  $\gamma$ -Irradiated and Silymarin group relative to  $\gamma$ -irradiated group.

**Table I.- Evaluation of total phenol contents and antioxidant properties of silymarin.**

Parameters	Silymarin Mean±SD (n=3)
Total phenol content (mg GAE /mg)	0.527±0.016
Total antioxidant activity (μmol/l)	139±10.5
DPPH scavenging activity ( mg/ml)	1.52±0.12

The value of GSH content and the activity of SOD and CAT of the liver of rats exposed to  $\gamma$ -radiation significantly increased as compared to the corresponding values of control and other groups. Rats received silymarin extracts and exposed to  $\gamma$ -radiation have exhibited an obvious elevation in the level of hepatic GSH and the activity of SOD and CAT compared to  $\gamma$ -Irradiated rats (Table II).

## DISCUSSION

The effort to develop radioprotectant agents has been initiated decades ago in order to protect human cells from radiation damage whereby synthetic and natural compounds have been studied (Ahmad *et al.*, 2013). In this study, the effectiveness of silymarin treatment in ameliorating the damage effects induced by gamma-irradiation was evaluated.

In this study, the results of total phenolic contents, high antioxidant activity and also DPPH scavenging activity recorded in silymarin can explain its antioxidant properties and indicated that is a powerful antioxidant herbal drug which can protect biological systems against the oxidative stress. Asghar and Masood (2008) reported that silymarin shows high antioxidant capacity mainly due to its phenolic compounds and inhibits lipid peroxidation

in plasma and RBC models and also suggested that silymarin may be used in preventing free radical-related diseases as a dietary natural antioxidant supplement.

In the present study, the elevation in the activity of the serum enzymes ALT, AST, ALP and GGT after gamma-irradiation exposure could indicate occurrence of liver injury (Makhlouf and Makhlouf, 2012). Ali *et al.* (2012) reported that the damage of cellular membranes of hepatocytes following exposure to ionizing radiation leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the aminotransferase activities in liver and blood serum.

On the other wise, treatment with oral silymarin extract against gamma radiation in this work efficiently trims down the elevated levels of serum biomarkers such as AST, ALT, ALP and GGT and these results in agreement with that of Naik *et al.* (2015). Banakar *et al.* (2004) found that the significant reduction in the level of liver enzymes as results of silymarin supplementation could be attributed to the ability of silymarin as potent antioxidant to prevent liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes into the serum.

The present data revealed that gamma radiation exposure (6 Gy) resulted in significant acceleration in the oxidation of lipid by elevating the level of hepatic MDA and XO activity. Elkady and Mohamed (2016) reported that the exposure to ionizing radiation increases the production of ROS and directs the irradiated cells into a state of oxidative stress that has been implicated in a variety of natural and pathological processes. Radiation induced radiolysis of water in the aqueous media of the cells which leads to production of hydroxyl radicals ( $\bullet$ OH) that interact with the polyunsaturated fatty acids in the lipid portion of biological membranes initiating the lipid peroxidation and finally damaged the cell membranes (Azab *et al.*, 2011). While, the observed decrease in the activities of hepatic GSH content and the activity of XDH, SOD and CAT in  $\gamma$ -Irradiated group in this study could be due to a feedback inhibition or oxidative inactivation of the enzyme protein caused by reactive oxygen species (ROS) generation, which in turn can impair the antioxidant defense mechanism, leading to an increased membrane LPO (Mihandoost *et al.*, 2014).

On the other hand, the group of rats that received silymarin (50 mg/kg/day) and exposed to gamma radiation showed an obvious reduction in hepatic MDA and XO activity with significant elevation in hepatic GSH content and the activity of SOD and CAT in compared to irradiated-rats. The results are in agreement with those of Kiruthiga *et al.* (2014) who found that silymarin administration normalized the activities of SOD, CAT,

**Table II.-** Effect of Silymarin extract administration and  $\gamma$ -irradiation exposure on the activities of serum enzymes (ALT, AST, ALP and GGT), hepatic MDA and xanthine oxidoreductase system (XO and XDH) and hepatic GSH and the activities of SOD and CAT.

Parameters	Control	Silymarin	Irradiated	Irr.+ silymarin
<b>Seral enzymes</b>				
AST (U/ml)	37.16±2.45 <sup>a</sup>	35.91±2.68 <sup>a</sup>	60.87±2.25 <sup>c</sup>	42.57±2.86 <sup>b</sup>
ALT (U/ml)	26.19±1.84 <sup>a</sup>	26.55±1.79 <sup>a</sup>	39.32±1.76 <sup>c</sup>	31.52±1.88 <sup>b</sup>
ALP (U/100ml)	8.66±1.48 <sup>a</sup>	8.81±1.65 <sup>a</sup>	15.29±1.51 <sup>c</sup>	11.25±1.59 <sup>b</sup>
$\gamma$ GT (U/ml)	4.17±1.32 <sup>a</sup>	4.28±1.41 <sup>a</sup>	6.61±1.57 <sup>c</sup>	5.70±1.57 <sup>b</sup>
<b>Hepatic lipid peroxide</b>				
MDA (n mol/ml)	196.11±4.36 <sup>a</sup>	189.52±3.42 <sup>a</sup>	365.89±4.25 <sup>c</sup>	251.20±4.93 <sup>b</sup>
XO (mU/mgprotein)	2.51±0.31 <sup>a</sup>	2.46±0.28 <sup>a</sup>	3.82±0.22 <sup>c</sup>	2.85±0.34 <sup>b</sup>
<b>Hepatic antioxidant</b>				
GSH (mg/g tissue)	28.17±2.12 <sup>a</sup>	28.77±1.95 <sup>a</sup>	16.35±1.73 <sup>b</sup>	26.37±1.92 <sup>a</sup>
SOD (U/mg protein)	44.96±2.12 <sup>a</sup>	45.65±2.07 <sup>a</sup>	29.86±1.88 <sup>c</sup>	40.14±1.76 <sup>b</sup>
CAT (U/g protein)	3.17±0.11 <sup>a</sup>	3.29±0.09 <sup>a</sup>	1.72±0.08 <sup>c</sup>	2.87±0.10 <sup>b</sup>
XDH (mU/mg protein)	3.34±0.26 <sup>a</sup>	3.53±0.23 <sup>a</sup>	1.61±0.21 <sup>c</sup>	2.91±0.22 <sup>b</sup>

Means in the same row with different superscripts are significantly different at ( $P < 0.05$ ), Values are expressed as mean  $\pm$  SD (n=7).

glutathione peroxidase (GSH-Px), glutathione reductase (GR), and glutathione-s-transferase (GST) as well as TBARS levels in hydrogen peroxide treated-erythrocytes. Kwon *et al.* (2013) obtained that silymarin enhances hepatic glutathione generation by elevating cysteine availability and inducing cysteine synthesis while inhibiting its catabolism to taurine.

Furthermore, Surai (2015) reported that the free radical scavenging and antioxidant properties of silymarin and silibinin are demonstrated by: (a) restoration of the endogenous antioxidant enzymes (SOD, CAT, GSH-Px, GR and GST) and non-enzymatic antioxidants (vitamins E and C) in the liver and other tissues of stressed animals; (b) increased intracellular concentration of GSH in liver and other tissues; (c) decreased lipid and protein oxidation, detected as reduced MDA/TBARS and carbonyl content; (d) decreased DNA fragmentation/damage and apoptosis and (e) reduced secretion of ALT, AST, ALP from the liver into the plasma due to hepatic injuries caused by ROS.

### CONCLUSION

In light of this study, the results suggested that the high antioxidant properties of silymarin may be related to its phenolic contents and free radical scavenging activity. Also, the results demonstrated the effectiveness of silymarin extract in the protection of liver damage induced by  $\gamma$ -radiation exposure. Thus, the use of natural products as silymarin to treated hepatotoxicity could be important in clinical radiation therapy to protect human cells from radiation damage.

### Statement of conflict of interest

Authors have declared no conflict of interest.

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