

## Studying the effect of silymarin against oxidative stress induced by chemotherapeutic protocol in breast cancer women

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### الخلاصة:

يحتل سرطان الثدي لدى النساء اهمية كبيرة من حيث كونه الاكثر شيوعا وانتشارا بين باقي الاورام السرطانية في مجتمعنا. يعتقد ان السبب الحقيقي وراء هذا المرض هو تغيرات في الحامض النووي منقوص الاوكسجين (دنا، DNA) نتيجة لافعال تأكسدية والتي تنتهي بانتاج الجذور الحرة. يعتبر خليط العلاج الكيماوي CAF من اهم الانظمة العلاجية المستعملة في سرطان الثدي لكن استخدامه مرتبط بتاثيرات سامة في أعضاء الجسم المختلفة من خلال آلية الاجهاد التأكسدي وأنتاج الجذور الحرة. مادة السيليمارين (silymarin) هي الخلاصة الجافة للنبته *silybium marianum* والمكونة من مركبات تستخدم سريريا كمضادات تأكسد ومكملات غذائية لفعاليتها في حماية بعض اعضاء الجسم (وبخاصة الكبد) من الاضرار النسيجية التي تسببها بعض الادوية والسموم. صممت هذه الدراسة السريرية لغرض بحث دور الحماية المحتمل لمادة السيليمارين المعطاة فمويا كمضاد أكسدة للوقاية من تسمم أعضاء الجسم الناشئ عن الاجهاد التأكسدي اثناء العلاج بالخليط الكيماوي (CAF) لدى النساء اللواتي يعانين من سرطان الثدي. اشتملت هذه الدراسة على 94 سيدة، 20 منهم كن أصحاء وأعتبرن كمجموعة سيطرة لاجل مقارنة التغيرات الحاصلة في معايير فرط الاكسدة، أما الـ 74 الباقية فكن مصابات بسرطان الثدي وخضعن لنمط الدراسة باستخدام طريقة التوزيع العشوائي حيث تم توزيعهن على ثلاثة مجاميع :

المجموعة (أ): تضمنت 24 مريضة استلمن الخليط الكيماوي (CAF) بواسطة الحقن الوريدي لثلاث جرع وبمعدل جرعة واحدة كل ثلاثة اسابيع .

المجموعة (ب): تضمنت 25 مريضة استلمن نفس الخليط الكيماوي (CAF) في المجموعة (أ) مضافا له مادة السيليمارين على شكل كبسول اعد خصيصا لهذا الغرض وبجرعة 210 ملغم/يوميا.

المجموعة (ج): تضمنت 25 مريضة استلمن نفس الخليط الكيماوي (CAF) في المجموعة (أ) مضافا له مادة السيليمارين على شكل كبسول اعد خصيصا لهذا الغرض وبجرعة 420 ملغم/يوميا.

أعتمدت طرق التقييم على قياس مستويات معايير فرط الاكسدة (MDA,GSH) في مصل الدم. تم قياس هذه المؤشرات للمجاميع المرضية الثلاثة قبل إعطاء العلاج و بعد 21, 42 و 63 يوماً من إعطاءه. أظهرت نتائج هذه الدراسة زيادة ملحوظة في عمليات الاجهاد التأكسدي لكافة المجاميع المرضية قبل أخذ العلاج وللمرضى الذين عولجوا بالخليط الكيماوي (CAF) متمثلة بالأرتفاع المعنوي لمستويات (MDA) والانخفاض المعنوي لمستويات (GSH) في مصل الدم. هذه الحالة من فرط الاكسدة عكست تماماً بعد اضافة السيليمارين لخليط ال(CAF) وبشكل يتناسب مع مقدار الجرعة وفترة العلاج. في ضوء النتائج التي افرزتها هذه الدراسة يمكننا الاستنتاج بان سرطان الثدي وخليط العلاج الكيماوي (CAF) يؤديان الى تكوين الجذور الحرة وبالتالي تضعيف الية الدفاع بواسطة مضادات الاكسدة مسببة تاثيرات سمية في أعضاء الجسم المتعددة, ولذلك فأن استخدام السيليمارين كمادة مضادة للاكسدة في هذه الدراسة السريرية بامكانها ان تقلل (اعتمادا على مقدار الجرعة وفترة الاستخدام) من مضاعفات عملية فرط الاكسدة وما ينتج عنها من ضرر في اجهزة الجسم.

### **Abstract**

Breast cancer became the commonest type of cancers among Iraqi women since the last two decades. The main underlying cause is thought to be DNA damage; much of which is oxidative in nature. CAF protocol (Cyclophosphamide + Adriamycin + 5-FU) associated with toxic effects in several body organs, mainly through production of free radicals and reactive oxygen species. Silymarin, the dried extract of a ripe seeds of the plant *silybium marianum*, was found to be a powerful antioxidant protective agent against toxin -induced tissue damage .The objective of this study is to evaluate the possible time and dose-dependent protective effect of the orally administered silymarin as antioxidant agent against oxidative stress induced by CAF protocol (mainly Adriamycin) in breast cancer women. This study included 94 subjects, 20 were healthy control women (for matching with oxidative stress markers) and 74 were breast cancer women that randomly distributed and allocated into three groups:

Group (A): Include 24 patients who received CAF protocol by I.V infusion once every 21 days and for 63days.

Group (B): Include 25 patients who received 210mg/day of along with the same CAF protocol of group (A);

Group (C): Include 25 patients who received 420mg/day of silymarin along with the same CAF protocol of group (A). Oxidative stress markers (MDA and GSH) were measured at baseline (zero time), after 21, 42, and 63 days of treatment for each patient group.

Our results showed an increase in the oxidative stress for both baseline patients and those treated with CAF protocol, manifested by significant increase in

MDA levels and GSH depletion, a state which is significantly reversed by use of silymarin, in a time and dose-dependent manner. Breast cancer and its antineoplastic CAF protocol produce free radicals which attenuate antioxidant defense mechanism of the body leading to several toxic effects on different body organs, so the use of antioxidant agent (silymarin) in this study may ameliorate, in a time and dose-dependant manner, the harmful effects of this protocol.

**Keywords:** Breast cancer, CAF protocol (cyclophosphamide+adriamycin+5-fluorouracil) Oxidative stress, silymarin.

### **Introduction:**

Breast cancer is a malignant tumor that has developed from cells of the breast. It occurs almost in women, but men can get it, too <sup>[1]</sup>. According to cancer registry section (Iraqi Cancer Board) Baghdad / Ministry of Health, breast carcinoma is the most common malignant tumor in Iraqi women and it comprise (31.3%) of all female malignant cases. <sup>[2]</sup> There are different kinds of risk factors. Some factors, like a person's age or race, can't be changed. Others are linked to cancer-causing factors in the environment. Still others are related to personal choices such as smoking, drinking, and diet <sup>[3]</sup>.

Breast cancer usually begin in the cells that line the ducts (ductal cancer), some begin in the cells that line the lobules (lobular cancer), and the rest in the other tissues. Breast cancer classified according to WHO <sup>[4]</sup> into: non-invasive (non-infiltrating) or in situ carcinoma; and invasive (infiltrating) carcinoma. The most beneficial and commonly used staging system of breast cancer is the American Joint Committee on Cancer (AJCC) classification, which is based on the tumor size (T), the status of regional lymph nodes (N), and the presence of distant metastasis (M) <sup>[5]</sup>. There are three major techniques that are commonly used to evaluate breast masses, excluding surgical procedures. These are: physical examination, mammography, and fine needle aspiration cytology (FNAC) <sup>[6]</sup>.

Local treatment (surgery or radiation) and systemic treatment (hormonal or chemotherapy) can be planned by number of ways. The most common sequence is: surgery - chemotherapy - radiation –and then hormonal therapy. Combination of two or three chemotherapeutic drugs is used in breast cancer to avoid drug resistance and for better response. Several such combination regimens or protocols are available, such as CAF (Cyclophosphamide + Adriamycin +5-FU), and CMF (Cyclophosphamide + Methotrexate+5-FU) <sup>[7]</sup>.

Adriamycin, or Doxorubicin, is a cytotoxic anthracycline antibiotic isolated from cultures of *Streptomyces peucetius* var. *caesius*; Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards; while 5-Fluorouracil (5-FU) is a pyrimidine analog (5-fluoro-2, 4(1*H*,3*H*)-pyrimidinedione). The use of these cytotoxic drugs against breast cancer is limited

by number of adverse effects and toxicities, including cardiotoxicity, nephrotoxicity, hepatotoxicity, neurotoxicity, myelosuppression and blood disorders. Much of these complications attributed to the induction of oxidative stress by CAF protocol (especially by doxorubicin)<sup>[8]</sup>.

Silymarin is a mixture of flavonolignans isolated from the ripe seeds of the medicinal plant *Silybum marianum* (milk thistle), comprised mainly of silybinin (SBN) (A,B) isosilybinin (ISBN), silychristin (SCN), silydianin (SDN) and taxifolin (TXF)<sup>[9]</sup>. Multiple biological effects of flavonoids have been described, including anti-inflammatory, anti-allergic, anti-haemorrhagic, anti-mutagenic, anti-neoplastic, and hepatoprotective activities<sup>[10]</sup>. Most flavonoids, including silibinin, can protect cells and tissues against the harmful effects of reactive oxygen species (ROS). Their antioxidant activity results from scavenging of free radicals and other oxidizing intermediates, chelation of iron or copper ions and inhibition of oxidases<sup>[11]</sup>. Flavonoids from *Silybum marianum* have been widely used for the treatment of liver disorders. In experimental animal models, they exerted not only a positive effect on intact liver cells or cells not yet irreversibly damaged, but also to stimulate their regenerative capacity after partial hepatectomy<sup>[12]</sup>. No adverse reactions have been reported due to silymarin use in rats or human; either with short term or in long-lasting therapy<sup>[13]</sup>.

The aim of the present study was to evaluate the possible time and dose-dependent effects of the orally-administered silymarin as a protective agent against oxidative stress which could be induced by CAF protocol in women with breast cancer.

### **Materials, Subjects and Methods:**

Chemicals, drugs, and instruments that were used in this study are mentioned with their manufactures and origins in (tables-1,2,3) respectively. This randomized clinical study was carried out on 80 female patients with different stages of breast cancer, all pass through one type of operative mastectomy and this is the first time they receive chemotherapy in their life's. These patients were with age range of 41-60 years (mean: 49± 1.5) and body weight range of 65-96 kg (mean: 76± 2.5). Certain exclusion criteria were followed to avoid interference of any other factors and include: those with history of previous chemotherapy, cardiac disorders, pregnant and breast feeding women, and those for whom any of CAF protocol components is contraindicated.

<b>Chemical</b>	<b>Manufacture</b>	<b>Origin</b>
5,5-dithio- bis (2-nitrobenzoic acid): DTNB	BDH chemicals, Ltd.	Poole, England
Thiobarbituric acid : TBA	BDH chemicals, Ltd.	Poole, England
Trichloroacetic acid:TCA	Merck chemical	Germany
Disodium hydrogen phosphate (Na <sub>2</sub> HPO <sub>4</sub> )	Fluka-Garantie	Switzerland
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	Merk, chemicals	Germany

**Table-1: Chemicals used in the study**

<b>Drug</b>	<b>Manufacture</b>	<b>Origin</b>
Doxorubicin	Ebewe Phrma	Austria
Cyclophosphamide	Baxter	Germany
5-Fluorouracil	Ebewe Phrma	Austria
Silymarin Standardized Powder	Luna Company	Egypt

**Table-2: Drugs used in the study**

<b>Instrument</b>	<b>Manufacture</b>	<b>Origin</b>
Cold Centrifuge -Universal 16A	Hettich	Germany
UV Spectrophotometer	Jenway 6300	U.K
pH meter pw 9420	Philips	Netherlan ds
Water Bath WB22	Memmert Lab.	Germany
Autovortex SA60	Stuart Scientific	U.K
Freezer	Hitachi	Japan
Sensitive balance	A and D company Ltd.	Japan
Micropipette	SLAMED	Germany

**Table-3: Instruments used in the study**

Only 74 female patients completed the study, others were excluded due to poor compliance with the follow up program. These patients were diagnosed and treated in Baghdad Teaching Hospital/ Department of Surgery/ Unit of Oncology under follow up of specialist doctors during the period from March 2009 to September 2009. Our patients were randomly allocated in three groups as follow:

Group (A): Include 24 patients who received CAF protocol (Cyclophosphamide 600 mg/m<sup>2</sup> + Adriamycin 60 mg/m<sup>2</sup>+ 5- FU 600 mg/m<sup>2</sup>) by intravenous infusion once every 21 days and for 63 days.

Group (B): Include 25 patients who received 210mg/day of silymarin (given as single dose in a capsule dosage form especially prepared for this purpose) along with same CAF protocol of group (A).

Group (C): Include 25 patients who received 420mg/day of silymarin (given as 210mg/12hour in a capsule dosage form especially prepared for this purpose) along with same CAF protocol of group (A).

Twenty healthy females were involved and considered as control group to compare the results of their oxidative stress markers with that of patient groups.

After over night fasting, venous blood (5 ml) was obtained from the forearm of each patient by vein puncture at a baseline before the initiation of therapy, after 21, 42 days of treatment and at the end of 63 days for all patient groups. Each blood sample was placed in EDTA-free tube to be centrifuged for 10 minutes at 3000rpm. Serum was then divided into several eppendorf tubes and kept frozen until time for the assay.

Malondialdehyde (MDA), the end product of lipid peroxidation, was analyzed according to the method of Buege and Aust<sup>[14]</sup> based on the reaction of MDA with thiobarbituric acid (TBA) to form a red chromophore, which can be quantitated spectrophotometrically. Total thiol group contents, which can be used as a marker for the reduced glutathione (GSH), were determined according to the method of Ellman<sup>[15]</sup>, where 0.5 ml of serum was added to 4.5 ml of 5,5 – dithiobis -(2nitro benzoic acid) DTNB reagent [0.1 mM DTNB in 0.1 M phosphate buffer pH =8]. The light absorbance of the solution at 412nm was measured after 2 minutes.

### **Statistical analysis:**

The results were expressed as mean ± standard error of mean (SEM). Student's paired t-test and ANOVA test were used to examine the degree of significance and *P* values < 0.05 were considered significant.

### **Results:**

At baseline, breast cancer produced significant elevation (*P*<0.05) in serum MDA levels (146%,158%,164%) for patients treated with CAF protocol, CAF protocol and 210 or 420 mg/day of silymarin, respectively compared to control

subjects(table 4). Further significant elevation ( $P<0.05$ ) in serum MDA levels was observed as a result of treatment with CAF protocol (43%,80%,94%) after 21,42 and 63 days, respectively compared with baseline. Significant reduction ( $P<0.05$ ) in serum MDA levels was observed for patients treated with CAF protocol and 210 mg/day of silymarin ( 21%, 41%, 58%) and those treated with CAF protocol and 420 mg/day of silymarin ( 23%, 43%, 60%) after 21,42, and 63 days of treatment, respectively compared with baseline values .There was significant difference ( $P<0.05$ ) in serum MDA levels for patients treated with CAF protocol and silymarin (210 or 420 mg/day) after the end of each treatment cycle compared with those received just CAF protocol. At the end of 63 days of treatment with CAF protocol and silymarin (210 or 420 mg/day), serum MDA levels were comparable ( $P>0.05$ ) to that of control values (table 4).

At baseline, breast cancer produced significant reduction ( $P<0.05$ )in serum GSH levels (40%,42%,41%) for patients treated with CAF protocol ,CAF protocol and 210 or 420 mg/day of silymarin, respectively compared to control subjects (table 5). Further significant reduction ( $P<0.05$ ) in serum GSH levels was observed as a result of treatment with CAF protocol (15%,31%, 48%) after 21,42 and 63 days, respectively compared with baseline. Significant elevation ( $P<0.05$ ) in serum GSH levels was observed for patients treated with CAF protocol and 210 mg/day of silymarin ( 15%, 32%, 50%) and those treated with CAF protocol and 420 mg/day of silymarin ( 15%, 33%, 68%) after 21,42, and 63 days of treatment, respectively compared with baseline values. There was significant difference ( $P<0.05$ ) in serum GSH levels for patients treated with CAF protocol and silymarin (210 or 420 mg/day) after the end of each treatment cycle compared with those received just CAF protocol. Meanwhile, the elevation in this parameter values was significant ( $P<0.05$ ) after 63 days of treatment with CAF protocol and 420 mg/day of silymarin (14%) compared with those on CAF protocol and 210 mg/day of silymarin. At the end of 63 days of treatment with CAF protocol and 420 mg/day of silymarin, serum GSH levels were comparable ( $P>0.05$ )tocontrolvalues (table5).

Group	Serum MDA ( $\mu\text{mol/l}$ )				
	Number of patients	Baseline	21 days post treatment	42 days post treatment	63 days post treatment
Control	20	0.998 $\pm$ 0.01			
CAF protocol	24	2.45 $\pm$ 0.47 <sup>a<math>\Psi</math></sup>	3.51 $\pm$ 0.12 <sup>b</sup>	4.42 $\pm$ 0.32 <sup>c</sup>	4.75 $\pm$ 0.27 <sup>c</sup>
CAF+Sily. (210mg/day)	25	2.57 $\pm$ 0.23 <sup>a<math>\Psi</math></sup>	2.03 $\pm$ 0.11 <sup>b<math>\dagger</math></sup>	1.52 $\pm$ 0.09 <sup>c<math>\dagger</math></sup>	1.07 $\pm$ 0.23 <sup>d<math>\dagger</math></sup>
CAF+Sily. (420mg/day)	25	2.63 $\pm$ 0.37 <sup>a<math>\Psi</math></sup>	2.02 $\pm$ 0.08 <sup>b<math>\dagger</math></sup>	1.49 $\pm$ 0.13 <sup>c<math>\dagger</math></sup>	1.04 $\pm$ 0.17 <sup>d<math>\dagger</math></sup>

**Table-4: Effects of treatment with 210 and 420 mg/day of silymarin on serum MDA levels in breast cancer patients treated with CAF protocol.**

Results were expressed as mean $\pm$  SEM

Results with non identical superscripts (a, b, c,d) within the same group were considered significantly different at  $P < 0.05$

$\dagger$  = Significant at  $P < 0.05$  as compared with CAF protocol values

$\Psi$  = Significant at  $P < 0.05$  as compared with control values

Group	Serum GSH ( $\mu\text{mol/l}$ )				
	Number of patients	Baseline	21 days post treatment	42 days post treatment	63 days post treatment
Control	20	0.321 $\pm$ 0.11			
CAF protocol	24	0.192 $\pm$ 0.02 <sup>a<math>\Psi</math></sup>	0.163 $\pm$ 0.04 <sup>b</sup>	0.132 $\pm$ 0.07 <sup>c</sup>	0.1 $\pm$ 0.03 <sup>d</sup>
CAF+Sily. (210mg/day)	25	0.185 $\pm$ 0.14 <sup>a<math>\Psi</math></sup>	0.213 $\pm$ 0.15 <sup>b<math>\dagger</math></sup>	0.244 $\pm$ 0.12 <sup>c<math>\dagger</math></sup>	0.277 $\pm$ 0.18 <sup>d<math>\dagger</math></sup>
CAF+Sily. (420mg/day)	25	0.188 $\pm$ 0.12 <sup>a<math>\Psi</math></sup>	0.217 $\pm$ 0.21 <sup>b<math>\dagger</math></sup>	0.250 $\pm$ 0.1 <sup>c<math>\dagger</math></sup>	0.315 $\pm$ 0.16 <sup>d<math>\dagger</math>*</sup>

**Table-5: Effects of treatment with 210 and 420 mg/day of silymarin on serum GSH levels in breast cancer patients treated with CAF protocol.**

Results were expressed as mean $\pm$  SEM

Results with non identical superscripts (a, b, c,d) within the same group were considered significantly different at  $P < 0.05$

$\dagger$  = Significant at  $P < 0.05$  as compared with CAF protocol values

\* = Significant at  $P < 0.05$  as compared with CAF protocol and silymarin (210mg/day) values

$\Psi$  = Significant at  $P < 0.05$  as compared with control values



**Discussion:**

Regarding oxidative stress and breast cancer relationship, the results presented in this study clarified that, in breast cancer patients and at baseline, serum MDA levels were significantly higher, while serum GSH levels were significantly lower, than that of control, which may be attributed to the overproduction of ROS, a state of systemic oxidative stress, and deficiency of antioxidant defense mechanism (table 4,5). These results support the oxidative stress hypothesis in carcinogenesis which was studied by Kumaraguruparan *et al.* and Khanzode *et al.*, who found an increase in lipid peroxidation in plasma and solid tumors<sup>[16,17]</sup>. Our results were consistent with those reported by Huang *et al.* and Gönenc *et al.*, who found significant increase in plasma MDA levels in cancer patients compared with normal subjects<sup>[18,19]</sup>. These results were also consistent with that recorded by Ray *et al.*, who studied lipid peroxidation, free radical production, and antioxidant status in breast cancer patients. The rate of superoxide ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ) production was significantly higher in breast cancer patients than normal<sup>[20]</sup>.

Concerning the effect of CAF protocol on oxidative stress, formation of free radicals via electron reduction is the mechanism supposed to induce cytotoxicity by doxorubicin<sup>[21]</sup>. Doxorubicin is quinone which can undergo one-electron reduction to semiquinone or two-electron reduction to the corresponding dihydroquinone derivatives. The semiquinone reacts rapidly with oxygen to generate  $O_2^{\cdot-}$ , which can be dismutated to  $H_2O_2$ , which in turn reacts in various ways to produce hydroxyl radicals. Hydroxyl radicals can react with polyunsaturated fatty acids, initiating a lipid-radical chain reaction and oxidative damage to cell membrane. Increased levels of ROS due to metabolism of doxorubicin have been detected by an increase in tissue MDA levels<sup>[22]</sup>. In the presence of transition metal ions, the chain reaction continues and free iron appears to play a particularly important role in doxorubicin- inducing lipid peroxidation. Without free iron, MDA formation is minimal and even a low concentration of free iron can lead to substantial MDA production<sup>[23]</sup>. Doxorubicin may act by transferring an electron directly to  $Fe^{3+}$  and the produced  $Fe^{2+}$  can reduce oxygen to hydrogen peroxide. Redox cycling of doxorubicin in this manner also generates free radical metabolites and ROS<sup>[24]</sup>.

The above hypothesis was supported by our study, in which there was significant elevation in serum MDA levels and significant reduction in serum GSH levels for patients who received doxorubicin (within CAF protocol) along treatment duration compared with their baseline values (table 4,5). These results were consistent with that reported by Eser Özl and Mustafa, who found that, in doxorubicin-treated animals, the MDA levels of kidney, lung, liver, and brain tissues were significantly increased, compared to control rats<sup>[25]</sup>. Veselina *et al.*

reported that plasma GSH levels were significantly reduced in lymphoproliferative cancer patients treated with doxorubicin <sup>[26]</sup>.

In addition to doxorubicin, cyclophosphamide (within CAF protocol) can also be considered as a source of ROS and contribute to the elevation of MDA levels and GSH depletion. Both cyclophosphamide and ifosfamide are metabolized to acrolein, which is responsible for the stimulation of oxidative stress, and then bladder toxicity. The co-administration of MESNA (sodium 2-mercaptoethane sulfonate), a sulphhydryl-containing compound which binds to acrolein, has reduced the incidence of haemorrhagic cystitis associated with ifosfamide and high dose of cyclophosphamide <sup>[27]</sup>.

Regarding the effect of silymarin on CAF-induced oxidative stress, silymarin antioxidant activity was studied by Rastogi *et al* on aflatoxin B<sub>1</sub>-induced lipid peroxidation in rat liver and kidney, where treatment with silymarin reversed the increase in lipid peroxide levels and increased the antioxidant levels near the normal values <sup>[28]</sup>. The antioxidant effect of silymarin was observed in rats intoxicated by acetaminophen and ethanol, which are oxidant stress inducers that produce marked MDA elevation and GSH depletion in the liver. Treatment with silymarin or silybinin was able to protect animals against oxidative stress produced in the liver by these chemicals <sup>[29]</sup>. Another study performed by Valenzuela showed that, when liver from rats pre-treated *in vivo* with intravenous silybinin (50 mg/kg), there was significant reduction in the oxygen consumption and MDA release stimulated by phenyl hydrazine, without any changes in GSH levels <sup>[30]</sup>.

Data reported in this study support the antioxidant effect of silymarin, where the administration of silymarin with CAF protocol significantly decreases serum MDA levels and increases serum GSH levels along the study compared with their baseline values, in a time and dose-dependent manner. After the end of treatment course, the levels of MDA and GSH were comparable to control values (table 4,5). Silymarin antioxidant activity can not be related only to the reduction of MDA production and GSH elevation. It has been reported that this effect included also improvement in the activities of many antioxidant enzymes including glutathione reductase (GSH-R), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) <sup>[31]</sup>.

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