Effect of Silymarin Against CAF Protocol Cardiotoxicity

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ABSTRACT:

BACKGROUND:
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, which is oxidative in nature. CAF protocol (Cyclophosphamide + Adriamycin + 5-FU) associated with toxic effects of heart and other organs, mainly through production of free radicals and reactive oxygen species. Silymarin, the dried extract of a ripe seeds of the plant *Silybum marianum*, was found to be a powerful antioxidant agent against toxin-induced tissue damage.

OBJECTIVE:
To evaluate the possible time and dose-dependent protective effect of the orally administered silymarin as antioxidant agent against oxidative stress-related cardiotoxicity induced by CAF protocol in breast cancer women.

PATIENTS AND METHODS:
74 breast cancer women randomly distributed and allocated into three groups:
Group (A): 24 patients received CAF protocol by I.V infusion once every 21 days and for 63 days.
Group (B): 25 patients received 210mg/day of silymarin along with the same CAF protocol of group (A).
Group (C): 25 patients received 420mg/day of silymarin along with the same CAF protocol of group (A).
Cardiac function enzymes (CK and LDH) were measured at baseline, after 21, 42, and 63 days of treatment.

RESULTS:
Levels of CK and LDH, which were significantly elevated by CAF protocol, showed significant reduction when silymarin used with CAF, in time and dose-dependent manner.

CONCLUSION:
The use of antioxidant agent (silymarin) in this study can ameliorate, in time and dose-dependant manner, the harmful cardiac effects of the oxidative stress that induced by breast cancer and its antineoplastic CAF protocol.

KEYWORDS: breast cancer, CAF protocol, cardiotoxicity, 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 (1). 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 (2,3). 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) (4,5). Techniques that are commonly used to evaluate breast masses are: physical examination, mammography and fine needle aspiration cytology (FNAC) (6).

Local treatment (surgery or radiation) and systemic treatment (hormonal or chemotherapy) can be planned by different sequences. 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) (7).

Doxorubicin (Adriamycin) 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-fluo-ro-2, 4(1H,3H)-pyrimidine dione). The use of these cytotoxic drugs against breast cancer is limited...
by several toxic effects, such as cardiotoxicity [tachyarrhythmia and ECG changes (acute); CHF and cardiomyopathy (chronic)], nephrotoxicity, hepatotoxicity, neurotoxicity, myelosuppression and blood disorders. Most of these complications attributed to the induction of oxidative stress by CAF protocol (especially doxorubicin) [9].

Silymarin is a mixture of flavonolignans isolated from the ripe seeds of the medicinal plant Silybum marianum (milk thistle), comprised mainly of silybinin, isosilybinin, silychristin, silydianin and taxifolin [9]. Multiple biological effects of flavonoids have been described, including anti-inflammatory, anti-allergic, anti-haemorrhagic, anti-mutagenic, anti-neoplastic, hepatoprotective and antioxidant activities [10]. Their antioxidant activity results from scavenging of free radicals and other oxidizing intermediates, chelation of iron or copper ions and inhibition of oxidases [11]. Flavonoids from Silybum marianum have been widely used for treatment of liver disorders [12]. No adverse reactions have been reported due to silymarin use in rats or human; either with short term or in long-lasting therapy [13]. 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-related cardiotoxicity which could be induced by CAF protocol in women with breast cancer.

PATIENTS AND METHODS:
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 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 and severe cardiac disorders.

Only 74 female patients completed this study, others were excluded due to poor compliance with the follow up program. The 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/m² + Adriamycin 60 mg/m² + 5-FU 600 mg/m²) by intravenous infusion once every 21 days and for 63 days.

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

Drugs used in this study were Doxorubicin, Cyclophosphamide, 5- Fluorouracil (Ebewe Pharma, Austria) and standardized powder of Silymarin (Luna comp. Egypt). Ready made kits (Random, UK) were used to determine serum creatine kinase (CK) and lactate dehydrogenase (LDH) activities by using UV Spectro-photometer (Jenway 6300, UK).

After over night fasting, venous blood (5 ml) was obtained from the forearm of each patient by vein puncture at baseline, 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 3000 rpm. Serum was then divided into several eppendorf tubes and kept frozen until time for assay of cardiac enzymes activities. Colorimetric determination of serum CK activities was based on that described by Oliver and Hess [14], and for serum LDH activities was based on that described by Wroblewski [15].

Regarding statistical analysis, our 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:
Table (1) revealed that the levels of serum CK significantly elevated (P<0.05) with CAF protocol (14%, 30%, 46%) after 21, 42 and 63 days of treatment respectively, compared with baseline data.

Patients who received CAF protocol with 210 mg/day of silymarin produced significant reduction (P<0.05) in serum CK levels (11%, 21%) after 42 and 63 days of treatment respectively, compared with baseline data.

Combination of CAF protocol and 420 mg/day of silymarin produced significant reduction (P<0.05) in serum CK levels (9%,18%,33%) after 21, 42 and 63 days of treatment respectively, compared with that of baseline. There was significant difference (P<0.05) in serum CK 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 reduction in this parameter values was significant (P<0.05) after 63 days of treatment with CAF protocol and 420 mg/day of silymarin (12%) compared with those on CAF protocol and 210 mg/day of silymarin.
### Table 1: Effects of treatment with 210 and 420 mg/day of silymarin on serum CK levels in breast cancer patients treated with CAF protocol.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum CK (U/L)</th>
<th>Number of patients</th>
<th>Baseline</th>
<th>21 days post treatment</th>
<th>42 days post treatment</th>
<th>63 days post treatment</th>
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<td>CAF protocol</td>
<td></td>
<td>24</td>
<td>85.18±11.36 a</td>
<td>97.16±15.18 b</td>
<td>110.70±16.43 c</td>
<td>124.27±13.11 d</td>
</tr>
<tr>
<td>CAF+Sily. (210mg/d)</td>
<td></td>
<td>25</td>
<td>84.04±10.24 a</td>
<td>81.23±13.07 b</td>
<td>74.92±9.17 c</td>
<td>66.37±14.78 d</td>
</tr>
<tr>
<td>CAF+Sily. (420mg/d)</td>
<td></td>
<td>25</td>
<td>87.89±12.35 a</td>
<td>80.35±11.06 b</td>
<td>72.26±10.44 c</td>
<td>58.77±8.67 d</td>
</tr>
</tbody>
</table>

Results were expressed as mean± standard error of mean (SEM). Results with non identical superscripts (a, b, c, d) within the same group were considered significantly different at P<0.05. †† Significant at P<0.05 as compared with CAF protocol value. * Significant at P<0.05 as compared with CAF protocol and silymarin (210mg/d) values. Kit normal values: 24-170 U/l

Table (2) revealed that the levels of serum LDH significantly elevated (P<0.05) with CAF protocol (7%, 15%, 17%) after 21, 42 and 63 days of treatment respectively, compared with baseline data. Patients who received CAF protocol with 210 mg/day of silymarin produced significant reduction (P<0.05) in serum LDH levels (8%, 15%) after 42 and 63 days of treatment respectively, compared with baseline data. Combination of CAF protocol and 420 mg/day of silymarin produced significant reduction (P<0.05) in serum LDH levels (5%, 12%, 19%) after 21, 42 and 63 days of treatment respectively, compared with that of baseline. There was significant difference (P<0.05) in serum LDH levels for patients treated with CAF protocol and silymarin (210 or 420 mg/d) after the end of each treatment cycle compared with those received just CAF protocol.

### Table 2: Effects of treatment with 210 and 420 mg/day of silymarin on serum LDH levels in breast cancer patients treated with CAF protocol.

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH (U/l)</th>
<th>Number of patients</th>
<th>Baseline</th>
<th>21 days post treatment</th>
<th>42 days post treatment</th>
<th>63 days post treatment</th>
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<tr>
<td>CAF protocol</td>
<td></td>
<td>24</td>
<td>176.40±16.99 a</td>
<td>188.32±18.25 b</td>
<td>201.93±15.46 c</td>
<td>205.74±23.12 d</td>
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<tr>
<td>CAF+Sily. (210mg/d)</td>
<td></td>
<td>25</td>
<td>174.54±13.82 a</td>
<td>171.88±21.36 b</td>
<td>160.08±9.78 b</td>
<td>147.93±10.66 d</td>
</tr>
<tr>
<td>CAF+Sily. (420mg/d)</td>
<td></td>
<td>25</td>
<td>178.76±17.94 a</td>
<td>170.65±19.61 b</td>
<td>158.11±11.05 c</td>
<td>144.04±8.75 d</td>
</tr>
</tbody>
</table>

Results were expressed as mean± standard error of mean (SEM). Results with non identical superscripts (a, b, c, d) within the same group were considered significantly different at P<0.05. †† Significant at P<0.05 as compared with CAF protocol values. Kit normal values: 80-190 U/l

**DISCUSSION:**

Reactive oxygen species (ROS) produced by doxorubicin through either enzyme-mediated cycling of semiquinone radical, or by forming doxorubicin-iron complex (16). In both pathways, molecular oxygen is reduced to superoxide anion (O2−), which is converted to other forms of ROS such as organic peroxides and hydroxyl radicals. These free radicals could then cause membrane and macromolecule damage, both of which lead to heart injury, an organ that has relatively low levels of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (17).

As a key intracellular target, cardiac mitochondria are sites of generation of highly reactive free radical intermediates, as well as sits for the interference with cellular Ca2+ regulation and bioenergetics, and its failure considered as a hallmarks of doxorubicin-induced cardiac toxicity (18). In our study, this mechanism of cardiac toxicity resulted in significant increase in serum CK and LDH activities (LDH
values were above their normal upper limits at the end of study) compared with their baseline values during doxorubicin treatment within CAF protocol along this study (tables 1,2), which suggest increasing leakage from mitochondria due to generation of free radicals that induce destructive myocardial injury leading to lysis in number of myocytes, as well as loss of cytoplasmic membrane integrity (19). Results of this study agree with those reported by Olson et al. and James et al., who found that serum CK and LDH activities were significantly increased by doxorubicin (20, 21).

High-dose intravenous cyclophosphamide infusion (120 to 240 mg/kg over 1 to 4 days) has been associated with CHF and death from hemorrhagic myocarditis. Treatment with protocols that involve doxorubicin and cyclophosphamide together may potentiate doxorubicin cardiotoxicity even if the dosage of doxorubicin is below the limit of 550 mg/m^2. 5-Fluorouracil cardiotoxicity is suspected to be mediated by coronary vasospasm and free radical damage to the myocardium (22). Recent study supports the hypothesis that 5-FU has direct endothelial toxicity and hypoxic resulting in thrombogenic effect by increasing fibrinopeptide in the circulation and release of vasoactive substances like endothelin (23).

Cardiotoxicity is a complication associated with several chemotherapeutic agents, including anthracyclines, cyclophosphamide and 5-fluorouracil. Some studies suggest that multiple drug regimens increase the risk of cardiotoxicity (24), and this consist with the results of the present study after administration of CAF protocol (tables 1,2), while others observed no apparent difference in the incidence of toxicity between monotherapies and polytherapies (25).

Regarding the liver, numerous in vivo and in vitro studies have been shown silymarin to be a potent lipid membrane antioxidant and one of the most potent scavengers of hydroxyl radical (26). In the present study, we try to expand the range of tissues which may be a candidate target for silymarin by providing more evidence for the importance of the antioxidant activity of silymarin in its suspected cardioprotective effect.

Silymarin attenuated the doxorubicin-induced elevation in malondialdehyde production (MDA) and massive depletion in reduced glutathione level (GSH) both in serum and myocardial tissue homogenate (27). This cardioprotective effect produced by silymarin may be due to membrane stabilizing effect and reduction in the process of lipid peroxidation. In addition, it may stimulate the activity of many antioxidant enzymes including glutathione peroxidase and catalase (28).

Doxorubicin binds to iron and the iron-doxorubicin complex catalyzes the free radical reactions (29). Silibinin may act as a potent iron-chelating agent, block the formation of oxygen free radicals, and prevent the formation of complexes between doxorubicin and iron and thus preventing doxorubicin cardiotoxicity (30).

Protein synthesis stimulation considered as an important step in repairing of tissue damage and is essential for restoring structural proteins and enzymes which are exposed to damage by toxic xenobiotics and/or their metabolites. Silymarin probably acts not only on the cell membrane, but also on the nucleus, where it increase ribosomal protein synthesis by stimulating RNA polymerase I and the transcription of rRNA (31).

Cardiac protection of silymarin in a dose-dependent manner was well documented in the present study, whereas increasing the dose can be reflected on better protection, manifested by more significant improvement in serum CK and LDH activities along this study, compared with their baseline values (tables 1,2).

Several studies found that serum LDH level was significantly reduced in human and animals received doxorubicin after chronic administration of vitamins E and C (32), while El-Habib et al. reported significant reduction in serum CK and LDH levels in rats treated with L-carnitine, as an antioxidant against doxorubicin damage (19). Results of this study clarified in tables (1,2) were also consistent with the previous studies which found that silymarin have shown protective effects on rat cardiomyocytes exposed to doxorubicin due to its antioxidant, iron chelating, and cell membrane stabilizing capacity (33). Treatment with probucol (the lipid-lowering agent with strong antioxidant properties) prevents 5-FU from inducing vascular endothelial damage by its thrombogenic effects (24). In our study, silymarin may have a similar cardioprotective effects of probucol against 5-FU cardiotoxicity as it possess both of these antioxidant and hypolipidemic properties.

CONCLUSION:

Breast cancer and its antineoplastic CAF protocol produce free radicals which attenuate antioxidant defense mechanism of the body leading to cardiotoxicity, so the use of antioxidant agent (silymarin) in this study can ameliorate, in time and dose-dependant manner, the harmful cardiac effects of this oxidative stress state.

REFERENCES:


