Matricaria chamomilla Extract Ameliorates Doxorubicin-Induced Cardiac Dysfunction in Male Rats

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Abstract: Doxorubicin (DOXO) is a highly effective antineoplastic drug against a wide range of malignant conditions. However, DOXO-generated oxidative stress promotes progression of cardiac toxicity. Chamomile (Matricaria chamomilla L.) possesses powerful therapeutic properties, as its extract contains many antioxidant constituents. This study aimed to investigate the possible protective effects of oral administration of chamomile flowers ethanolic extract (CFE) against DOXO-induced cardiomyopathy in male rats. The cardiac toxicity induced in rats by single intraperitoneally (i.p) injection of DOXO at a dose of 20 mg/kg body weight (b.wt.), CFE was given orally by gavage (p.o) at a dose level of 400 mg/kg b.w. daily starting seven days prior DOXO or saline injection and continued until the end of the experiment (10 days). Male rats (n= 40) (200-230 g) were divided equally into four groups as follows: Control group, DOXO group, CFE group and CFE + DOXO group. Cardiac functions, inflammatory mediators, cardiac enzymatic and non-enzymatic antioxidants were measured. As well as histological examination of heart tissues were examined. The results revealed that DOXO produced severe cardiotoxicity as indicated by a significant increase in cardiac enzyme activities of lactate dehydrogenase (LDH) and total creatine phosphokinase (CKP), as well as cytokine IL-1β in the plasma of rats. This was accompanied by significant increase in cardiac malondialdehyde (MDA) and total nitric oxide (NO). Besides, DOXO significantly reduced cardiac content of reduced glutathione (GSH) and enzymatic activity of superoxide dismutase (SOD) compared with control rats. Histopathological examination of heart tissue showed that DOXO induced degenerative changes in heart tissues and focal necrosis of cardiac myocytes associated with inflammatory cells infiltration and intermuscular oedema. Administration of CFE prior DOXO could restore the enzyme activities to near normal values and ameliorate all the studied biochemical markers, as well as protect cardiac tissues from histological alterations. These findings suggested that CFE ameliorated DOXO-induced cardiotoxicity, which could be attributed to inhibition of MDA formation and protection against antioxidants depletion via antioxidative and anti-inflammatory potential. In conclusion, the use of CFE has a protective role in the abatement of DOXO-induced cardiac toxicity.

Key words: Cardiotoxicity . Doxorubicin . Oxidative stress .Matricaria chamomilla . Rats.

INTRODUCTION

Anthracycline is the best known of the chemotherapeutic agents [1]. Doxorubicin (DOXO) is an antitumor antibiotic of the anthracycline group. It has been shown to be highly effective anti-neoplastic drugs in the treatment of several adult and pediatric cancers, such as soft tissue tumors, solid tumors, leukemia, lymphomas, breast cancer and a variety of malignant disorders [2]. Cardiotoxicity of chemotherapeutic agents includes a direct effect of the drug on the heart and an indirect effect due to enhancement of haemodynamic flow alterations or thrombotic events [3]. Unfortunately, the clinical use of DOXO is associated with severe cytotoxic side effects [4, 5]. Cardiotoxicity may occur in >20 % of patients treated with DOXO. The probability of developing cardiomyopathy is largely dose-dependent, even at low doses, thus hampered the successful use of
the drug [6, 7]. Swain et al. [8] demonstrated that DOXO administration induces left ventricular dysfunction and increased myocardial passive stiffness in rats. The mechanism of DOXO-induced cardiotoxicity is most likely mediated by the formation of an iron–anthracycline complex that generates free radicals, which in turn, causes diverse oxidative damage on critical cellular components and membrane lipids [9, 10]. Peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of DOXO [11]. Therefore, minimizing toxicity remains one of the major barriers to receiving a drug. Chamomile (*Matricaria chamomilla* L.) family Asteraceae is a reputed medicinal and aromatic plant used in traditional medicine [12]. It is most popular consumed as an herbal drink all over the world [13]. Chamomile has many pharmaceutical properties as anti-inflammatory, immunomodulatory and antitumor [14, 15], as well as antioxidant, antiplatelet and chemopreventive [16, 17]. Chamomile extraction contains many bioactive antioxidant constituents [16]. The proposed hypothesis was that, if DOXO cardiotoxicity is related to free radicals formation and lipid peroxidation, so antioxidants may protects against DOXO-induced toxicities in hearts.

Therefore, the present study aimed to investigate the possible protective effects of chamomile (*Matricaria chamomilla* L.) flower extract on DOXO-induced cardiac toxicity in rats. In addition, the mechanism by which CFE induced cardiac has been also discussed.

**MATERIALS AND METHODS**

**Chemicals:** Adriablastina (10 mg Adriamycin hydrochloride), Pharmacia Italia (S.P.A. Italy) was used in this study. All other chemicals and reagents used in this study with analytical grade were purchased from Sigma-Aldrich (St. Louis, MO) Chemical Co.

**Plant Material:** Chamomile (*Matricaria chamomilla* L.), a member of *Asteraceae* family was purchased as crude dried material from Medicinal and Aromatic Plants Research Department, Horticultural Research Institute, Agricultural Research Center, Giza, Egypt.

**Animals:** Forty male albino rats with a weight of 200-230 g were used in this experiment. Rats were fed standard laboratory casein diet according to Reeves et al. [18] and given water *ad libitum*. They were housed under controlled environmental conditions (12 h light/12h dark regular cycle in partially humid), left to accommodate one week before experimental use. The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996).

**Preparation of Plant Flower Ethanolic Extract:** The dried flowers of *chamomile* were grounded to a fine powder. 1000 g of the powder was then soaked in 70% ethanol (1.5L) with daily shaking and kept in refrigerator for three days [19]. The extraction procedure was repeated twice using the same powder. The infusions filtered from each extraction were mixed and the excess solvent was evaporated under reduced pressure, using a rotary evaporator apparatus (Switzerland) attached with vacuum pump. Each 100g of dried powder yield (16.15g) ethanol extract, which was stored at 4°C till used later in the experimental procedure.

**Experimental Design:** After the acclimatization period (one week), rats were randomly divided into four groups each of 10 rats. Control group (-ve); a volume of distilled water equal to CFE was given to rats by intragastric gavage tube 7 days before intraperitoneally (i.p.) injected with a single dose of saline and daily thereafter throughout the study (10 days). DOXO group i.p. injected with a single dose of 20 mg/kg DOXO dissolved in saline, which is well documented to induce acute cardiotoxicity [20]. CFE group received CFE suspended in distilled water orally at a dose level of 400 mg/kg based on the results of previous study [21], for 7 days before saline i.p. injection and daily thereafter throughout for 10 days. DOXO + CFE group received CFE as in CFE group before DOXO injection and daily thereafter throughout the study (10 days) Pretreatment with CFE for 7 days prior to administration of DOXO was chosen based on a preliminary experiment that revealed pretreatment with CFE just prior to DOXO showed no benefits.

**Sample Collection and Biochemical Analysis:** At the end of experiment orbital blood samples were collected under light ether anesthesia using heparinized microcapillaries. Heart specimens were collected. Plasma was separated for biochemical analysis. Frozen heart specimens were used for enzymatic and non-enzymatic antioxidant measurement. Formalin fixed heart specimens were used for histopathological examination.
Determination of Cardiac Anti-Oxidants: Heart tissues from each group were washed with ice-cold phosphate buffer saline (PBS). Tissues homogenized (20% w/v) was prepared by sonication in ice-cold PBS (pH 8.0, 0.01 M) using a polytron homogenizer (pt 3100) (five cycles of 10 s at 3000 rpm). Aliquots were prepared and used for the assessment of different cardiac antioxidants [25].

Preparation of Cardiac Aliquots: Proteins were precipitated by centrifugation after addition of an equal volume of a 20% trichloroacetic acid solution (TCA). Aliquots were prepared and used for the assessment of different cardiac antioxidants; enzymatic and non-enzymatic.

Determination of Plasma Cardiac Biomarker Enzymes: Plasma total lactate dehydrogenase (LDH) and total creatine phosphokinase (CPK) activities were determined using commercial Stanbio kits (San Antonio, TX, USA). Total LDH activity was assessed according to the method of Henry [22] and Buhl and Jackson [23]. The method depends on the reaction of lactate with NAD. The NADH formed is measured at 340 nm using Shimadzu spectrophotometer UC 1201 (Japan). The increase in absorbance is measured at 1-min intervals for 3 min. Plasma total LDH activity was calculated as units per liter (U/L). Total CPK activity was determined according to the method of Abbot et al. [24]. The method based on transphosphorylation of ADP to ATP through a series of coupled enzymatic reactions; NADH produced is directly proportional to the CPK activity. The increase in absorbance at 1-min intervals was recorded for 3 min at 340 nm. Plasma total CPK activity was calculated as units per liter (U/L).

Determination of Plasma Interlukine-1 beta (IL-1β): Plasma level of IL-1β was measured by Assay Max interleukin-1 beta (enzyme-linked immunosorbent assay) ELISA kits (provided by R & D systems, Inc., Germany) using antibody specific for IL-1β. Cytokine concentrations are calculated using a standard purified recombinant cytokines according to manufacturers’ instruction.

Determination of Reduced Glutathione (GSH): Reduced glutathione (GSH) was determined according to the method described by Ellman [26]. The procedure is based on the reduction of bis-(3-carboxy- 4-nitrophenyl) disulfide reagent by SH group to form 2-nitro-5-mercaptopbenzoic acid, which has an intense yellow color. Briefly, aliquots of the supernatant were then mixed with eight times volume of 0.3M sodium phosphate and an equal volume of DTNB solution prepared by dissolving 4.0 mg of DTNB in 10ml of 1.0% trisodium citrate solution. The absorbance was measured spectrophotometrically at 412 nm and GSH levels were calculated with reference to the standards [27].

Determination of Lipid Peroxides (Measured as MDA): Malondialdehyde, a reactive aldehyde that is a measure of lipid peroxidation, was determined according to the method of Uchiyama and Mihara [28]. The adducts formed following the reaction of tissue homogenate with thiobarbituric acid in boiling water bath, were extracted with n-butanol. The difference in optical density developed at two distinct wavelengths; 535 nm and 525 nm was a measure of the tissue MDA content. Tissue MDA content was expressed as nmol/g tissue.

Determination of Superoxide Dismutase (SOD): Cardiac activity of SOD was assessed according to the method of Marklund [29]. It simply resides on computing the difference between autooxidation of pyrogallol alone and in presence of the cytosolic fraction that contains the enzyme. Changes in the absorbance at 420 nm were recorded at 1-min interval for 5 min. Enzyme activity was expressed as U/g wet tissue.

Determination of Cardiac Total Nitric Oxide (NO): Cardiac total nitric oxide (NO) was determined using a commercial kit from R & D Systems (MN, USA) according to the method of Miles et al. [30]. This assay determines total NO based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by a colorimetric detection of nitrite as an azo dye product of the Griess Reaction. The Griess Reaction is based on the two-step diazotization reaction in which acidified NO₃ produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylenediamine to form the chromophoric azoderivative, which absorbs visible light at 540 nm. Cardiac NO level was expressed as ìmol/g wet tissue.

Histopathological Examination of Heart Tissue Sections: Formalin (10%) fixed heart sections were embedded in paraffin wax, serially sectioned (3-5 µm thickness) and stained with haematoxylin and Eosin (H&E) for histopathological examination.
Statistical Analysis: Results were expressed as (mean ± SD). Data were analyzed statistically by analysis of variance, for statistical significance using L.S.D. test, one way ANOVA, post hoc multiple comparisons.

RESULTS

Cardiac Biomarker Enzymes: Administration of CFE for 10 consecutive days had no significant effect on the plasma activity of total LDH (Fig. 1). Doxorubicin in a single dose induced significant (p<0.001) elevation plasma LDH activity by about 174.05% compared to normal value in control group. Administration of CFE for 7 consecutive days prior DOXO injection and daily thereafter throughout for 10 days induced significant (p<0.001) reduction in LDH activity by 59.39% compared to rats injected with DOXO alone. LDH activity in pretreated CFE group prior DOXO injection was, however, significantly different (p<0.05) from control value. As shown in Fig. 2, plasma level of CKP was significantly (p<0.001) elevated 72 hr after DOXO injection amounting to 438.14% as compared to the value in the control rats. Pretreatment with CFE caused significant (p<0.001) decrease in CPK plasma activity compared to DOXO injected rats, the CKP value in CFE+DOXO group reaching 118.01 % of the control value.

Cardiac Anti-Oxidants: The effect of CFE and/or DOXO on cardiac content of GSH and MDA and enzyme activity of SOD in male rats are compiled in Table 1. Oral administration of CFE had no effect on cardiac content of reduced GSH and lipid peroxides (MDA), as well as SOD compared to their respective values in control group. Injection of rats with a single dose of DOXO caused a significant (p<0.001) increase in cardiac MDA content reaching to 190.67% accompanied by significant (p<0.001) decrease in cardiac GSH content and SOD activity reaching to 44.61% and 29.06%, respectively of the control values. Pretreatment of DOXO-injected rats with CFE 7 days before DOXO injection and daily thereafter throughout for 10 days significantly (p<0.001) prevent this significant elevation.

Table 1: Effect of CFE and/ or DOXO on cardiac content of GSH, MDA and enzyme activity of cardiac SOD in male rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>GSH (µmol/g tissue)</th>
<th>MDA (nmol/g tissue)</th>
<th>SOD (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.43 ± 0.24</td>
<td>197.39 ± 14.12</td>
<td>53.37 ± 5.29</td>
</tr>
<tr>
<td>DOXO</td>
<td>1.53 ± 0.15***</td>
<td>376.36 ± 20.66***</td>
<td>15.51 ± 1.39***</td>
</tr>
<tr>
<td>CFE</td>
<td>3.54 ± 0.18</td>
<td>181.98 ± 9.96</td>
<td>54.77 ± 4.62</td>
</tr>
<tr>
<td>CFE+DOXO</td>
<td>3.15 ± 0.31**</td>
<td>207.27 ± 19.98***</td>
<td>48.32 ± 4.40***</td>
</tr>
</tbody>
</table>

Each value represents the mean of 10 rats ± SD. * Significant difference from control group). ** Significant difference between DOXO group and DOXO group pretreated with CFE. ’p < 0.05, ”p < 0.01 and ***p < 0.001

Cytokine Interlukine-1 Beta (IL-1β): The concentration of IL-1 β in control, DOXO, CFE and CFE+DOXO groups are presented in Fig. 3. The results revealed that, DOXO caused significant (p<0.001) elevation in IL-1β concentration amounting to 219.93% as compared to the level in the control rats. There was insignificant (p>0.05) change between CFE and control group. Pretreatment of DOXO-injected rats with CFE (400 mg/kg) 7 days before DOXO and daily thereafter throughout for 10 days resulted in significant (p<0.001) decrease in plasma IL-1β concentration compared to DOXO injected rats.

Fig. 1: Effect of CFE and/ or DOXO on plasma LDH activity in male rats

Each value represents the mean of 10 rats ± SD. *Significant difference from control group. **Significant difference between DOXO and DOXO pretreated with CFE group. ’p < 0.05, ”p < 0.01 and *** < 0.001
Fig. 2: Effect of CFE and/or DOXO on plasma CPK activity in male rats

Each value represents the mean of 10 rats ± SD. *Significant difference from control group. **Significant difference between DOXO and DOXO pretreated with CFE group. ™ p < 0.05, ℹ ™ p < 0.01 and ™™™ p < 0.001

Fig. 3: Effect of CFE and/or DOXO on plasma IL-1β concentration in male rats

Each value represents the mean of 10 rats ± SD. *Significant difference from control group. **Significant difference between DOXO and DOXO pretreated with CFE group. ™ p < 0.05, ℹ ™ p < 0.01 and ™™™ p < 0.001

Fig. 4: Effect of CFE and/or DOXO on cardiac NO content in male rats

Each value represents the mean of 10 rats ± SD. *Significant difference from control group. **Significant difference between DOXO and DOXO pretreated with CFE group. ™ p < 0.05, ℹ ™ p < 0.01 and ™™™ p < 0.001
counteracted the DOXO-induced elevation in cardiac MDA and reduction in cardiac reduced GSH level and SOD activity as compared with untreated DOXO group. Unfortunately, pretreated rats did not show significant reduction in MDA as well as significant increase in GSH and SOD values compared to their respective control values as there were significant ($p<0.05$) changes as compared with control rats. As shown in Fig. 4, DOXO injection caused a significant ($p<0.001$) increase (206.21%) in cardiac NO content compared to the control value. Pretreatment of DOXO-injected rats with CFE resulted in significantly decrease cardiac NO content by 45.05% with respect to untreated DOXO group value, there was significant ($p<0.001$) difference compared to untreated DOXO-injected rats.

**Histopathological Results:** Examination of heart tissue sections of the different studied groups is shown in Fig. 5. Heart tissue sections of control rats showed normal histopathological structure of cardiac myocytes (Fig. 5A).
Heart tissue sections of rats from DOXO injected group showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (Fig. 5B-1), congestion of myocardial blood vessels and Zenker’s necrosis of cardiac myocytes (Fig. 5B-2 and B-3) and intermuscular oedema (Fig. 5B-4). Heart tissue section of CFE rats showed normal cardiac myocytes (C). Pretreatment of DOXO- injected rats with CFE (400 mg/kg b.wt/day) 7 days before DOXO injection and daily thereafter throughout for 10 days showed apparently no histopathological changes (Fig. 5D-1), expects some sections which showed slight congestion of cardiac blood vessels (Fig. 5D-2).

DISCUSSION

Doxorubicin is one of the most effective and widely used chemotherapeutic agents against leukemia, lymphomas and various solid tumors of the lung, breast, thyroid and ovary [31-34]. Almost all clinically used antitumor drugs exhibit toxic side effects affecting heart function. The congestive heart failure induced by DOXO is proven refractory to commonly used therapeutic procedures [35]. Increased oxidative stress and antioxidant deficit have been suggested to play a major role in doxorubicin induced cardiomyopathy, congestive heart failure due to multiple treatments with DOXO [36]. The precise cellular mechanisms responsible for this chronic cardiotoxicity of DOXO remain enigmatic; however, the antitumor activity of DOXO is likely to be distinct from the mechanism of its cardiotoxicity [37]. Nevertheless, no one single chemical has been proven to reduce the deleterious action of DOXO. To counteract the cardiotoxic effect of anthracyclines, the uses of antioxidants have been suggested. Therefore, the search for an effective and safe antagonist of DOXO cardiac toxicity remains a critical issue in both cardiology and oncology.

Antioxidant compounds are very promising in animal models of anthracycline-induced cardiotoxicity. Chamomile is one of the most widely used as medicinal plants. It has been included in the pharmacopoeia of 26 countries. Main constituents of the flowers include several phenolic compounds, primarily the flavonoids apigenin, quercetin, patuletin, luteolin and their glucosides (e.g., apigenin 7-glucoside) [16]. Chamomile flower extract has cyclooxygenase-2 (COX-2) inhibitor with anti-inflammatory activity [38], antioxidant, antiplatelet and chemporeventive action [15, 16]. The main objective of this study was to investigate the possible protective effect of the chamomile flower ethanolic extract (CFE) as antioxidant and anti-inflammatory against DOXO-induced cardiotoxicity and in male rats. In this study DOXO markedly increased the enzymatic activities of plasma LDH and CPK. Actually, these enzymes are considered as important markers of early and late cardiac injury especially during clinical follow-up of doxorubicin therapy [39]. Many previous studies have demonstrated similar elevations in cardiac enzymes activities in rats following challenge with a single cumulative dose of doxorubicin (15-20 mg/kg) [20, 40, 41]. Leakage of cardiac enzymes directly correlates to ultra-structure damage of heart tissues. Doxorubicin-induced cardiomyopathy is mainly attributed to increase oxidant production in heart. It may undergo a one-electron reduction through a metabolic activation by NADPH-cytochrome P-450 reductase. This reduction leads to the formation of the free radical semiquinone, which in turn can produce a variety of active ROS/RNS, including H2O2, •OH and ONOO [42]. These species can attack the cardiomyocyte membrane, damage several macromolecular cellular components, cause protein and lipid peroxidation and consequently lead to cardiomyocyte apoptosis or death [43]. This effect would compromise the cellular integrity and potentially account for the leakage of heart enzymes, LDH and CPK, through the membranes and increase their serum activity.

In the present results, DOXO-induced cardiomyopathy was also associated with increase pro-inflammatory cytokine IL-1β. These results agree with a previous study that revealed cardiac inflammation and dysfunction after DOXO injection [44]. Anthracyclines produce a drug-related systemic inflammation which has been found to be mediated by interleukins [45]. In particular, interleukin-1beta (IL-1beta) has been implicated in this mechanism. Doxorubicin induces a systemic increase in IL-1beta and other inflammatory cytokines, chemokines and growth factors including TNF-alpha, IL-6, CXCL1/Gro-alpha, CCl/MCP-1, granulocyte colony stimulating factor and CXCL10/IP-10. The IL-1beta release required the expression of caspase-1, NLRP3 and the adaptor protein ASC indicating that inflammation is mediated by the NLRP3 inflamasome [46, 47].

The molecular mechanisms by which anthracyclines trigger IL-1beta release are not completely understood, however the undesirable consequences of anthracyclines due to their inflammatory activity that complicate chemotherapy may be reduced by suppressing the actions of IL-1β. Our results indicated that CFE
pretreatment mitigated the increase of the cardiac enzyme activities. This was manifested by the significant decrease in plasma LDH and CPK activities and pro-inflammatory cytokine IL-1β compared with untreated DOXO group. The protective effect of CFE may be attributed to the antioxidant components of CFE such as apigenin 7-O-glucoside and various acylated derivatives of apigenin 7-O glucoside [48, 49]. Moreover, chamomile is one of the richest natural sources of a flavone apigenin (840 mg/100 g). Apigenin affects a number of cellular processes including cell signaling enzymes, pathways and gene expression [50], regulation of cell membrane transport [51], cytokine production and the inflammatory response [52]. Smolinski and Pestka [53] stated that pro-inflammatory cytokine production is inhibited in mice pretreated with apigenin extracted from chamomile. Evidence supporting the potential mechanisms involved in these processes may explain the role of chamomile in chemoprevention as an inhibitor of cell proliferation and oncogene expression and as an anti-inflammatory agent.

In the present study, DOXO injection caused significant increase in MDA level accompanied by significant decrease in cardiac GSH content and SOD activity, which are in agreement with the previous studies [25, 54, 55]. The elevated MDA level might be attributed to DOXO-mediated oxidative stress. The first targets of DOXO-mediated free radicals damage are various cellular membranes, which are rich in lipids prone to peroxidation. This radical’s damage results in production of many relatively stable and highly toxic aldehydes, such as MDA. These aldehydes can easily diffuse within the cell, or even cross the plasma membrane and attack macromolecular targets far from where they were generated and thus act as “second cytotoxic messengers” [56]. Moreover, the overproduction of ROS, caused by DOXO administration, can account for the decrease in GSH content and a depletion of SOD in cardiac muscle, as these species are detoxified by endogenous antioxidants mainly GSH causing their cellular stores to be depleted [57, 58]. In addition, the observed decrease in the antioxidant enzyme activities could be explained on the basis of their exhaustion in combating the previously observed oxidative stress [59]. Results of the present study revealed that, CFE pretreatment attenuated DOXO-induced cardiotoxicity. This was manifested by the significant decrease in cardiac MDA content, accompanied by significant increase in cardiac GSH content and SOD activity. The protective effect of CFE is associated with its antioxidant properties, as it acts as ROS scavenger and an inhibitor of MDA. These results are in agreement with those obtained by Sebai et al. [60], who found that chamomile extract produced a significant protection against castor oil-induced oxidative stress, due to its potent antioxidant activity. Moreover, Chamomile recutita flowers ethanolic extract showed potent antioxidant and hepatorenal protective effects in diabetic rats [61]. Chamomile recutita extract contains high levels of polyphenolic compounds such as coumarins and flavonoids. The major secondary components from M. chamomile belong to three different chemical classes’ sesquiterpenes, coumarins and flavonoids [62]. Therefore, chamomile is one of the richest sources of antioxidants which could explain cardioprotective effect against DOXO-induced oxidative stress. Nitric oxide synthase (NOS) catalyzes the production of nitric oxide (NO). Inducible nitric oxide synthase (iNOS) is expressed by vascular endothelial cells and smooth muscle cells in response to cytokines. NO produced by iNOS is implicated in inflammatory diseases [63].

The present results revealed a marked increase in cardiac NO level in those rats received DOXO, while pretreatment of DOXO-injected rats with CFE resulted in significant decrease in cardiac NO. This finding is in harmony with previous study by Saad et al. [64], which used a model of DOXO-induced cardiomyopathy similar to that used in our study. The increase in NO level can be explained on the basis of the ability of DOXO to mediate induction of nitric oxide synthase (NOS) expression and, hence, NO release in heart [65]. Previous studies suggested that stimulation of endothelial cells with calcium mobilizing agents activates and dissociates the membrane-bound eNOS [66]. Because DOXO-induced toxicity is mediated by intracellular H2O2 as well as calcium influx, DOXO injection causes an increase in eNOS transcription and protein activity in aortic endothelial cells and thus NO synthesis. Food and phytochemicals exerts NO- suppressing activity via three different pathways: The blocking of iNOS expression, inactivation of iNOS catalytic function and the scavenging NO; while NO suppressing effect primarily through regulation of cellular iNOS expression. Chamomilla extract inhibits the formation of free radicals and scavenge the reactive oxygen metabolites via its high antioxidant compounds [67].

The previously mentioned biochemical results produced by DOXO injection are confirmed by the obtained cardiac histopathological results which showed
that DOXO induced rat’s serious morphological changes in the myocardium. The present results are in agreement with previous studies that revealed, the disturbance in oxidant-antioxidant systems results in tissue injury [68-69]. Doxorubicin-induced oxidative stress is generally attributed to the formation of the highly reactive hydroxyl radical (OH*), stimulator of lipid peroxidation and source for destruction and damage to the cell membrane [70]. Clinical and experimental evidences suggested the involvement of free radicals mediated oxidative process in the pathogenesis of DOXO induced cardiomyopathy [71]. Pretreatment of DOXO-injected rats with CFE 7 days before DOXO and daily thereafter could ameliorate the morphological changes in cardiac tissues. This could be attributed to its potent antioxidant action resulting in alleviation of altered metabolic status and membrane stability. *Matricaria recutita* provides cardioprotection due to its antioxidant and anti-peroxidative properties of its flavonoids and phenolic compounds which may be responsible for cardioprotective activity [72].

**CONCLUSION**

The present study demonstrates that CFE protects against DOX-induced cardiotoxicity in rats as evidenced by improved enzymes cardiac markers and restoration of the oxidant/antioxidant status as well as restoring histopathological changes. This may be attributed, at least in part, to its antioxidant and inflammatory activity. Although the exact mechanisms remain to be clarified, CFE could be an effective course of adjuvant therapy to enhance therapeutic efficacy and to lessen DOXO toxicity in clinical chemotherapy. Therefore, further studies are needed to reveal the exact underlying mechanism(s) of how CFE may prevent cardiotoxicity induced by anthracyclines drugs.

**REFERENCES**


