

Evaluation of the Potential Cardioprotective Activity of Some Saudi Plants against Doxorubicin Toxicity

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Doxorubicin (DOX) is an anthracycline antibiotic widely used as a chemotherapeutic agent in the treatment of several tumours. However, its cardiac toxicity limits its use at maximum therapeutic doses. Most studies implicated increased oxidative stress as the major determinant of DOX cardiotoxicity. The local Saudi flora is very rich in a variety of plants of quite known folkloric or traditional medicinal uses. *Tribulus macropterus* Boiss., *Olea europaea* L. subsp. *africana* (Mill.) P. S. Green, *Tamarix aphylla* (L.) H. Karst., *Cynomorium cocineum* L., *Cordia myxa* L., *Calligonum comosum* L' Hér, and *Withania somnifera* (L.) Dunal are Saudi plants known to have antioxidant activities. The aim of the current study was to explore the potential protective effects of methanolic extracts of these seven Saudi plants against DOX-induced cardiotoxicity in rats. Two plants showed promising cardioprotective potential in the order *Calligonum comosum* > *Cordia myxa*. The two plant extracts showed potent *in vitro* radical scavenging and antioxidant properties. They significantly protected against DOX-induced alterations in cardiac oxidative stress markers (GSH and MDA) and cardiac serum markers (CK-MB and LDH activities). Additionally, histopathological examination indicated a protection against DOX-induced cardiotoxicity. In conclusion, *C. comosum* and *C. myxa* exerted protective activity against DOX-induced cardiotoxicity, which is, at least partly, due to their antioxidant effect.

Key words: Saudi Plants, Doxorubicin, Cardioprotection

Introduction

Doxorubicin (DOX) is one of the most effective antitumour antibiotics belonging to the class of anthracyclines, but its use is limited by high incidence cardiotoxicity (Hortobágyi, 1997). With the increasing use of DOX, an acute cardiotoxicity has been recognized as a severe complication of DOX chemotherapy (Doroshov, 1991). Although numerous mechanisms have been proposed, most studies supported that increased oxidative stress, along with a reduction in the levels of antioxidants, plays a key role in the pathogenesis of

DOX-induced cardiomyopathy (Yen *et al.*, 1996). Therefore, the use of natural or synthetic antioxidants might protect from oxidative stress caused by DOX and other cytotoxic drugs (Bristow *et al.*, 1981). Diets rich in fruits and vegetables have been associated with decreased risks of several chronic diseases, such as coronary heart disease (Hertog *et al.*, 1993). These protective effects have been attributed partly to the various antioxidant compounds, *e.g.* vitamins C and E, β -carotene, and polyphenolics (Diplock *et al.*, 1998). Several compounds with antioxidant activities are known

to against DOX-induced toxicities. Lycopene, a carotenoid occurring in tomatoes (Yilmaz *et al.*, 2006), and gingerols in *Zingiber officinale* (Ajith *et al.*, 2008) were found to protect against DOX-induced nephrotoxicity. The antioxidant properties of flavonoids were shown to reduce DOX toxicity due to their ability to scavenge free radicals (Vaclavikova *et al.*, 2008).

The local Saudi flora is very rich in a variety of plants of quite known folkloric or traditional medicinal uses. Amongst such plants, *Tribulus macropterus* Boiss., *Olea europaea* L. subsp. *africana* (Mill.) P. S. Green, *Tamarix aphylla* (L.) H. Karst., *Cynomorium coccineum* L., *Cordia myxa* L., *Calligonum comosum* L' Hér, and *Withania somnifera* (L.) Dunal are known to have antioxidant activities (Al-Awadi *et al.*, 2001; Badria *et al.*, 2007; Nawwar *et al.*, 2009; She *et al.*, 2009; Bharavi *et al.*, 2010; Kadry *et al.*, 2010; Omar, 2010).

In continuing our interest in the evaluation of the biological activities of Saudi plants (Abdel-Sattar *et al.*, 2010a, b; Elberry *et al.*, 2010; Salah El Dine *et al.*, 2011), the present study was designed to screen the methanolic extracts of the selected seven Saudi plants for a potential protective effect against DOX-induced cardiotoxicity in rats.

Material and Methods

Plant materials and extract preparation

The seven plant species were collected from different localities of Saudi Arabia from May to June 2009 (Table I). A herbarium specimen of each collected species was prepared and kept at the herbarium of the Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. The collected plants were identified by the staff of the Department of Biology, Faculty of Science, King Abdulaziz University. Plant materials were air-dried, ground, and kept in dark, air-tight closed containers until extraction.

Each plant sample (500 g) was extracted (2 x 2000 ml) with methanol (100%) using an Ultra-Turrax T50 high-speed homogenizer (IKA, Staufen, Germany). The solvent was evaporated under reduced pressure, and the dried extracts were kept at 4 °C.

Chemicals

DOX was obtained as doxorubicin hydrochloride (2 mg/ml) from EBWE Pharma (Unterach, Austria). 4-Aminoantipyrine, ammonium thiocyanate, ascorbic acid, bovine serum albumin, butylated hydroxyanisole (BHA), carboxymethylcellulose (CMC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ellman's reagent, ferric chloride, glutathione reduced form (GSH), glutathione reductase, methanol (MeOH), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), potassium ferricyanide, 1,1,3,3-tetraethoxypropane, and trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of the highest grade commercially available.

In vitro studies

DPPH radical scavenging activity

The free radical scavenging activity of the extracts was determined by the DPPH method as described by Braca *et al.* (2001). An aliquot (0.1 ml) of each concentration of plant extracts (50, 100, or 200 µg/ml) was added to 3 ml of a 0.004% MeOH solution of DPPH and kept in the dark. Absorbance at 517 nm was determined after 30 min, and inhibition was calculated as follows: inhibition (%) = $[(A_0 - A_1)/A_0] \cdot 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample containing the extract/standard. The DPPH solution without sample was used as control. All tests were run in triplicate and the results averaged. Ascorbic acid was used as positive control.

Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each extract (50, 100, or 200 µg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 200 x g for 10 min. An aliquot (2.5 ml) of the supernatant was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally the absorbance was measured at 700 nm against a blank. Ascorbic acid was used as a positive control.

Total antioxidant activity

Antioxidant activity was assessed using the linoleic acid system (Osawa and Namiki, 1981). An extract (0.2 ml, 100 μ g/ml) of each sample was added to a solution of linoleic acid (0.13 ml), 99.8% ethanol (10 ml), and 0.2 M phosphate buffer (pH 7.0, 10 ml). The total volume was adjusted to 25 ml with distilled water. The reaction mixture was incubated at 40 °C, and the degree of oxidation was measured according to the thiocyanate method (Misuda *et al.*, 1966) by sequentially adding ethanol (10 ml, 75% v/v), ammonium thiocyanate (0.2 ml, 30% w/v), sample solution (0.2 ml), and ferric chloride (0.2 ml, 20 mM in 3.5% HCl) solution. After stirring for 3 min, the peroxide value was determined by reading the absorbance at 500 nm, and the percentage inhibition of linoleic acid peroxidation, $100 - [(\text{absorbance increase of sample} / \text{absorbance increase of control})] \cdot 100$, was calculated to express the antioxidant activity. All tests and analyses were run in triplicate and the results averaged. BHA was used as a positive control.

In vivo studies

Animals and experimental protocol

A total of 192 male Wistar rats, weighing 250–300 g, were used in the study in accordance with the guidelines of the Biochemical and Research Ethical Committee at King Abdulaziz University. Animals were housed in a well-ventilated, temperature-controlled room at (22 ± 3) °C with a 12 h/12 h light-dark cycle. Food, normal rat chow, and water were provided *ad libitum*. Care was taken to avoid stressful conditions. All experimental procedures were performed between 8 and 10 a.m.

Rats were randomly assigned to 16 groups (12 rats each). Group I received CMC [0.5%, 1 ml/ (200 g body weight \cdot d)] orally for 10 consecutive days. Group II received CMC orally for 10 consecutive days and a single dose of DOX [15 mg/kg body weight, intraperitoneally (i.p.)] on day 7 (Fadillioglu *et al.*, 2004). Groups III through IX received only the methanolic extracts of *T. macropterus*, *O. europaea*, *T. aphylla*, *C. coccineum*, *C. myxa*, *C. comosum*, and *W. somnifera*, respectively, suspended in 0.5% CMC (100 mg/kg body weight, orally once daily for 10 consecutive days). Groups X through XVI received each of the respective seven plant extracts combined with DOX in the previously mentioned doses; each

plant extract was administered for 10 consecutive days, and DOX was administered once on day 7.

Twenty-four h after the last plant extract or CMC treatment (day 11), rats were anesthetized with thiopentone (35 mg/kg, i.p.). Blood samples were collected by orbital puncture in serum-separating tubes. The blood was centrifuged at 3000 \times g for 15 min to separate the sera that were kept at -70 °C until biochemical analyses. The abdomen of each rat was opened and hearts were rapidly dissected out, washed in ice-cold isotonic saline, and blotted between two filter papers. Four hearts from each group were fixed in 10% formalin for histopathological examination, and the remaining hearts from each group were homogenized in ice-cold 0.1 M potassium phosphate buffer (pH 7.4) and stored at -70 °C for subsequent analyses.

Cardiac biochemical assays

The cardiac GSH content was determined according to the method of Adams *et al.* (1983), and values are expressed as μ mol/g protein. Lipid peroxidation products were determined by measuring the malondialdehyde (MDA) content in tissue homogenates according to the method of Mihara and Uchiyama (1978). The MDA content was measured spectrophotometrically at 532 nm and calculated based on a standard curve using 1,1,3,3-tetraethoxypropane as a standard. Values are expressed as nmol/g protein.

Serum biochemical assays

Creatine kinase isoenzyme-MB (CK-MB) and lactate dehydrogenase (LDH) activities were determined according to standard methods using diagnostic kits from BioSystems S. A. (Barcelona, Spain). The CK-MB activity was assayed by measuring the rate of NADPH formation at 340 nm (Young, 1990). The LDH activity was determined by measuring the rate of the reduced form of nicotinamide adenine dinucleotide (NADH) formation at 340 nm (Lorentz *et al.*, 1993).

Determination of protein content

The protein content of cardiac tissue homogenates was determined by the Lowry protein assay using bovine serum albumin as the standard (Lowry *et al.*, 1951).

Histopathological study

Heart sections from all rats were fixed in 10% buffered formalin, then embedded in paraffin. Sections of tissues were cut at 5 μ m thickness,

mounted on slides, stained with hematoxylin and eosin (H&E), and examined under a light microscope (Olympus BX-50, Olympus Corporation, Tokyo, Japan).

Statistical analysis

Data are expressed as means \pm SEM. Results were analysed using one-way ANOVA followed by Tukey-Kramer multiple comparisons tests using Software GraphPad InStat, Version 3 (GraphPad Software Inc., La Jolla, CA, USA). Differences were considered significant at $p < 0.05$.

Results

Extraction yields

Table I shows the yields of the dried methanolic extracts in percent of the dry plant material.

In vitro studies

DPPH radical scavenging activity

The antioxidant activities of the different methanolic extracts were first examined by exploring the scavenging of the stable DPPH free radical. All extracts were examined at concentrations of 50, 100, and 200 $\mu\text{g/ml}$, which were chosen based on experience reported in the literature (Rajkumar *et al.*, 2011). All extracts were able to reduce the violet stable radical DPPH to the yellow diphenylpicrylhydrazine in a dose-related manner, in the following order, at the highest concentration used: *W. somnifera* (57.1%), *O. europaea* (51.7%), *C. myxa* (62.6%), and *C. comosum* (91.2%). The other extracts had weaker scavenging activities below 50% (Table II).

Reducing power

Table III shows the Fe^{3+} -reducing power of methanol extracts compared to ascorbic acid. Like the free radical scavenging activity, the reducing power of methanolic extracts increased with increasing amounts of sample. The potency of the extracts were in the following order: *C. comosum* and *C. myxa* $>$ *W. somnifera* $>$ *O. europaea*. The other extracts exhibited lower activities.

Total antioxidant activity

The antioxidant activity of each of the plant extracts was assessed using the linoleic acid system. Fig. 1 indicates that all extracts tested at the concentration of 100 $\mu\text{g/ml}$ exhibited obvious antioxidant activity. The antioxidant activities of the extracts roughly paralleled their reducing activities.

In vivo studies

Effect of the methanolic extracts on tissue and serum cardiac markers

Table II. DPPH radical scavenging activity of the methanolic plant extracts.

Plant	Inhibition (%)		
	50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$
<i>Tribulus macropterus</i>	20.3 \pm 0.20	25.2 \pm 0.40	28.3 \pm 0.30
<i>Olea europaea</i>	34.6 \pm 1.20	43.2 \pm 1.10	51.7 \pm 1.40
<i>Tamarix aphylla</i>	27.2 \pm 0.50	31.3 \pm 0.30	38.2 \pm 0.60
<i>Cynomorium coccineum</i>	30.2 \pm 0.70	37.6 \pm 0.40	44.2 \pm 0.80
<i>Cordia myxa</i>	46.3 \pm 1.90	53.6 \pm 0.40	62.6 \pm 2.10
<i>Calligonum comosum</i>	70.6 \pm 3.10	82.3 \pm 4.20	91.2 \pm 5.30
<i>Withania somnifera</i>	41.5 \pm 1.10	50.3 \pm 1.40	57.1 \pm 1.30
Ascorbic acid	50.3 \pm 2.20	64.6 \pm 2.70	75.3 \pm 3.10

Data are the mean \pm SEM of 6 replicates.

Table I. Plants, their families, part used, place of collection, herbarium specimen number (SN), and yields of methanol extraction.

Plant	Family	Part used ^a	Place of collection	SN	Yield (g/100 g)
<i>Tribulus macropterus</i> Boiss.	Zygophyllaceae	AP	Jeddah	TM1162	10
<i>Olea europaea</i> L. subsp. <i>africana</i> (Mill.) P. S. Green	Oleaceae	AP	Al-Baha	OE1125	26.6
<i>Tamarix aphylla</i> (L.) H. Karst.	Tamaricaceae	AP	Al-Maddinah	TA1205	18
<i>Cynomorium coccineum</i> L.	Cynomoriaceae	AP	Al-Maddinah	CC1206	28
<i>Cordia myxa</i> L.	Boraginaceae	F	Al-Maddinah	CM1225	40
<i>Calligonum comosum</i> L' Hér	Polygonaceae	AP	Umm Lajj	CC1226	15
<i>Withania somnifera</i> (L.) Dunal	Solanaceae	AP	Al-Taif	WS1154	7.7

^a AP, aerial parts (vegetative stage); F, fruits.

Table III. Reducing power of the methanolic plant extracts.

Plant	Absorbance at 700 nm		
	50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$
<i>Tribulus macropterus</i>	0.26 \pm 0.02	0.30 \pm 0.01	0.43 \pm 0.02
<i>Olea europaea</i>	0.64 \pm 0.05	0.81 \pm 0.04	1.43 \pm 0.09
<i>Tamarix aphylla</i>	0.36 \pm 0.02	0.50 \pm 0.04	0.87 \pm 0.07
<i>Cynomorium coccineum</i>	0.52 \pm 0.03	0.67 \pm 0.07	1.12 \pm 0.09
<i>Cordia myxa</i>	0.87 \pm 0.09	1.38 \pm 0.08	1.88 \pm 0.10
<i>Calligonum comosum</i>	1.33 \pm 0.07	1.57 \pm 0.08	1.88 \pm 0.09
<i>Withania somnifera</i>	0.76 \pm 0.07	1.13 \pm 0.09	1.71 \pm 0.10
Ascorbic acid	1.53 \pm 0.06	1.87 \pm 0.08	2.87 \pm 0.09

Data are the mean \pm SEM of 6 replicates.

Table IV. Effects of the methanolic plant extracts on cardiac tissue oxidative stress (GSH and MDA) and serum cardiac markers (CK-MB and LDH activities).

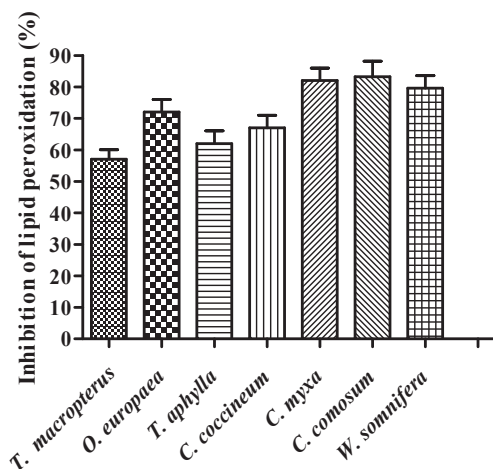
Plant	Cardiac tissue		Serum cardiac markers	
	GSH [$\mu\text{mol/g}$ protein]	MDA [nmol/g protein]	CK-MB [IU/mg protein]	LDH [IU/mg protein]
Control	4.42 \pm 0.13	55.3 \pm 1.80	93.0 \pm 6.70	122 \pm 9.80
<i>Tribulus macropterus</i>	4.51 \pm 0.11	55.1 \pm 3.20	95.3 \pm 4.76	121 \pm 6.07
<i>Olea europaea</i>	4.0 \pm 0.15	52.8 \pm 2.60	97.0 \pm 5.75	124 \pm 4.31
<i>Tamarix aphylla</i>	4.11 \pm 0.14	53.7 \pm 3.10	91.2 \pm 3.95	119 \pm 5.13
<i>Cynomorium coccineum</i>	4.21 \pm 0.18	55.9 \pm 2.50	98.8 \pm 4.24	120 \pm 4.69
<i>Cordia myxa</i>	4.25 \pm 0.23	50.7 \pm 2.40	88.3 \pm 3.78	122 \pm 4.36
<i>Calligonum comosum</i>	4.52 \pm 0.17	56.2 \pm 1.90	95.3 \pm 4.01	122 \pm 4.08
<i>Withania somnifera</i>	4.82 \pm 0.22	54.8 \pm 2.30	93.0 \pm 4.42	116 \pm 4.47

Data are the mean \pm SEM of 6 rats.

Table IV shows the effects of the plant extracts (100 mg/kg body weight) on the oxidant status in cardiac tissues as well as the serum activities of CK-MB and LDH as markers of cardiac injury. All extracts did not significantly affect these parameters as compared to the control (saline-treated) group. Thus, the tested extracts were not cardiotoxic under our experimental conditions.

Oxidant status in cardiac tissues

The data in Table V indicate that treatment of rats with DOX resulted in severe oxidative stress in cardiac tissues as is evidenced by significant GSH depletion as well as accumulation of MDA. Pre- and co-treatment with all examined plant extracts significantly protected against DOX-induced GSH depletion and lipid peroxidation. It

Fig. 1. Antioxidant activity of the methanolic plant extracts (100 $\mu\text{g/ml}$).

is noteworthy that *C. comosum* restored the GSH and MDA levels to control values. This clearly highlighted the excellent antioxidant and protective properties of this plant.

Activity of cardiac serum markers

The activities of serum CK-MB and LDH (Table V) were assessed as markers of cardiac injury. DOX insult resulted in significant elevation of both enzyme activities as compared to the control group. *T. macropterus* failed to provide any significant protection. LDH activity was restored to almost control level in the combined DOX + *C. comosum* group. Although not normalized, CK-MB activity was significantly reduced by the extracts in the following order *C. comosum* > *C. myxa* > *W. somnifera* > *O. europaea* > *C. coccineum* > *T. aphylla* as compared to the DOX group.

Table V. Effect of methanolic plant extracts on doxorubicin (DOX)-induced alterations in cardiac tissue oxidative stress (GSH and MDA) and serum cardiac markers (CK-MB and LDH activities).

Plant	Cardiac tissue		Serum cardiac markers	
	GSH [$\mu\text{mol/g protein}$]	MDA [nmol/g protein]	CK-MB [IU/mg protein]	LDH [IU/mg protein]
Control	4.42 \pm 0.13	55.3 \pm 1.80	93.0 \pm 6.70	122 \pm 9.80
DOX	1.93 ^a \pm 0.06	154 ^a \pm 5.30	189 ^a \pm 10.0	211 ^a \pm 10.3
DOX + <i>Tribulus macropterus</i>	2.62 ^{a,b} \pm 0.12	125 ^{a,b} \pm 4.50	158 ^a \pm 8.50	186 ^a \pm 8.60
DOX + <i>Olea europaea</i>	3.11 ^{a,b} \pm 0.15	91.5 ^{a,b} \pm 5.50	145 ^{a,b} \pm 7.50	164 ^{a,b} \pm 6.40
DOX + <i>Tamarix aphylla</i>	2.76 ^{a,b} \pm 0.13	114 ^{a,b} \pm 4.90	153 ^{a,b} \pm 8.20	176 ^a \pm 6.80
DOX + <i>Cynomorium coccineum</i>	2.91 ^{a,b} \pm 0.14	102 ^{a,b} \pm 4.30	148 ^{a,b} \pm 7.10	167 ^{a,b} \pm 8.10
DOX + <i>Cordia myxa</i>	3.62 ^{a,b} \pm 0.14	80.0 ^{a,b} \pm 3.70	137 ^{a,b} \pm 6.40	160 ^{a,b} \pm 7.20
DOX + <i>Calligonum comosum</i>	3.94 ^b \pm 0.13	73.4 ^b \pm 3.30	122 ^{a,b} \pm 5.80	148 ^b \pm 6.40
DOX + <i>Withania somnifera</i>	3.36 ^{a,b} \pm 0.12	86.2 ^{a,b} \pm 3.50	141 ^{a,b} \pm 8.30	163 ^{a,b} \pm 6.20

Data are the mean \pm SEM of 6 rats.

^a $p < 0.05$ vs. corresponding control group; ^b $p < 0.05$ vs. corresponding DOX group.

Histopathological study

Cardiotoxicity induced by DOX was further assessed by the examination of hematoxylin- and eosin-stained sections. Hearts from the control group and groups treated with plant extracts only (groups I and III–IX) showed regular cell distribution and normal myocardium architecture (Fig. 2).

Histological examination of hearts from DOX-treated rats revealed severe cytoplasmic vacuolar degeneration, interstitial edema and fibrotic bands (Fig. 3A). Administration of the methanolic extracts in addition to DOX improved the histopathological pattern in the following order: *C. comosum* (Fig. 3G) > *C. myxa* (Fig. 3F) > *O. europaea* (Fig. 3C) > *T. aphylla* (Fig. 3D) as compared to the DOX group. Extracts from *T. macropterus*, *C. coccineum*, and *W. somnifera* (Figs. 3B, E, and H) had a marginal effect on the histopathological pattern provoked by DOX administration.

Discussion

The genus *Tribulus* belongs to the family Zygophyllaceae. One of the most important species of the genus *Tribulus* is *T. terrestris*, which is traditionally used for the treatment of various ailments. It is used in the treatment of impotence to increase sexual activity, in cardiac diseases, and has antimicrobial, cytotoxic, and anthelmintic properties (Kostova and Dinchev, 2005). Previous phytochemical investigations of the genus *Tribulus* revealed the presence of steroidal glycosides (Sun *et al.*, 2002), flavonoids (Annapurna *et al.*,

2009), and alkaloids (Wu *et al.*, 1999) as the major bioactive phytoconstituents. The presence of steroidal saponins and flavonoids provided a basis for examining the Saudi *T. macropterus* species for potential protection against DOX cardiotoxicity in rats. In comparison to the other examined extracts, this plant showed the least radical scavenging and antioxidant *in vitro* activity. Although the plant significantly improved the cardiac oxidative status as indicated the GSH and MDA levels, it did not exhibit significant reduction in the activity of serum marker enzyme activities (CK-MB and LDH). This indicates that cardioprotection requires more than high antioxidant levels. Another possibility is that the doses or the regimens used in the current study were inappropriate for demonstrating potential cardioprotective activities of the plant.

The aerial parts of *O. europaea* were found to contain radical scavenging, reducing, and antioxidant activities. Further, the *in vivo* studies indicated a potential to mitigate DOX-induced cardiotoxicity as is evident from guarding against GSH depletion and MDA accumulation in cardiac tissues. These results are consistent with the observed significant reduction of the two serum cardiac markers. However, only a slight protection was afforded by *O. europaea* against histological changes. Yet, the current results are promising and worth further investigations. This finding is in accord with the reported cardiac protective properties of olive oil (Waterman and Lockwood, 2007). The observed effects of *O. europaea* extract were attributed mainly to its major component

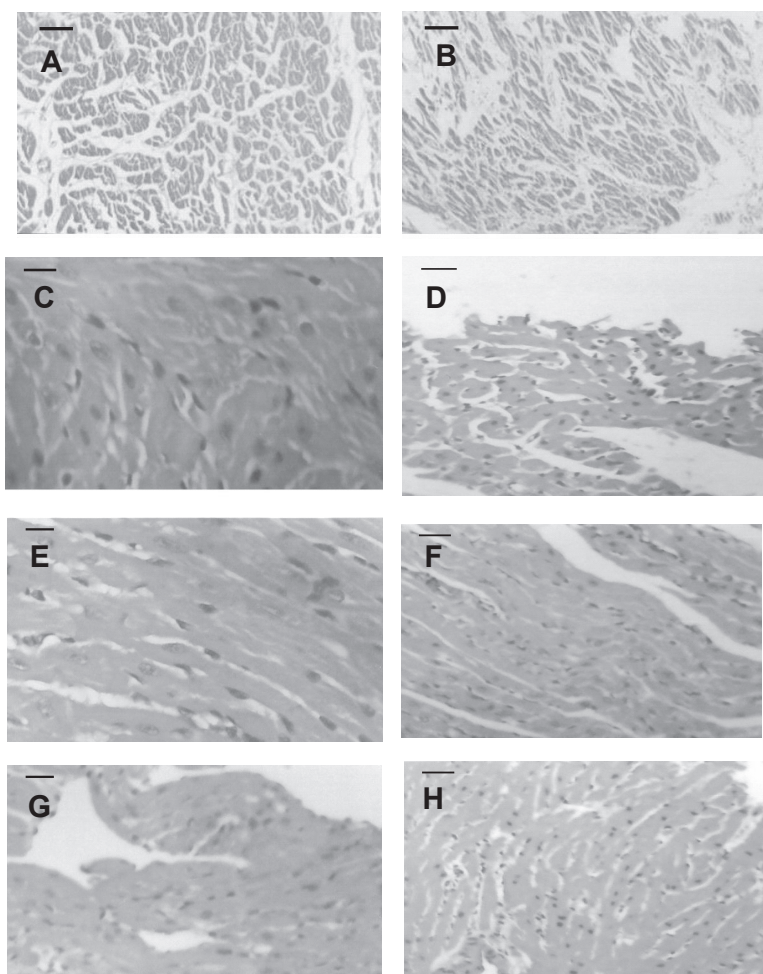


Fig. 2. Effect of methanolic plant extracts on cardiac histological pattern. Heart sections showing normal histological pattern from (A) control group rats which received carboxymethylcellulose (CMC; 0.5%) for 10 consecutive days and rats which received plant extracts (100 mg/kg) suspended in CMC (0.5%) for 10 consecutive days: (B) *T. macropterus*; (C) *O. europaea*; (D) *T. aphylla*; (E) *C. coccineum*; (F) *C. myxa*; (G) *C. comosum*; (H) *W. somnifera*. Scale bar, 50 μ m.

oleuropein, a phenolic antioxidant compound which is present in high concentration in the different organs of the olive tree and is effective against acute DOX cardiotoxicity through suppression of oxidative and nitrosative stress (Andreadou *et al.*, 2007).

T. aphylla is known for its high content of phenolic compounds, and the extract of its flowers exhibited a distinct radical scavenging effect and improved the viability of human keratinocytes (HaCaT cells) (Nawwar *et al.*, 2009). In the present study, the methanolic extract of *T. aphylla* exhibited radical scavenging and antioxidant ac-

tivity and significantly protected against DOX cardiotoxicity. However, the *T. aphylla* extract barely protected against DOX-induced cardiac histological changes. This strengthens our previous suggestion that antioxidation alone may not be sufficient to provide cardioprotection.

C. coccineum L. has been reported to have a hypotensive effect (Ikram *et al.*, 1978); anthocyanins appeared to be the major active constituents (Harborne *et al.*, 1994). These compounds attracted increasing interest, because of their relatively high intake in humans and their positive health effects. Choi *et al.* (2007) reported a cytoprotec-

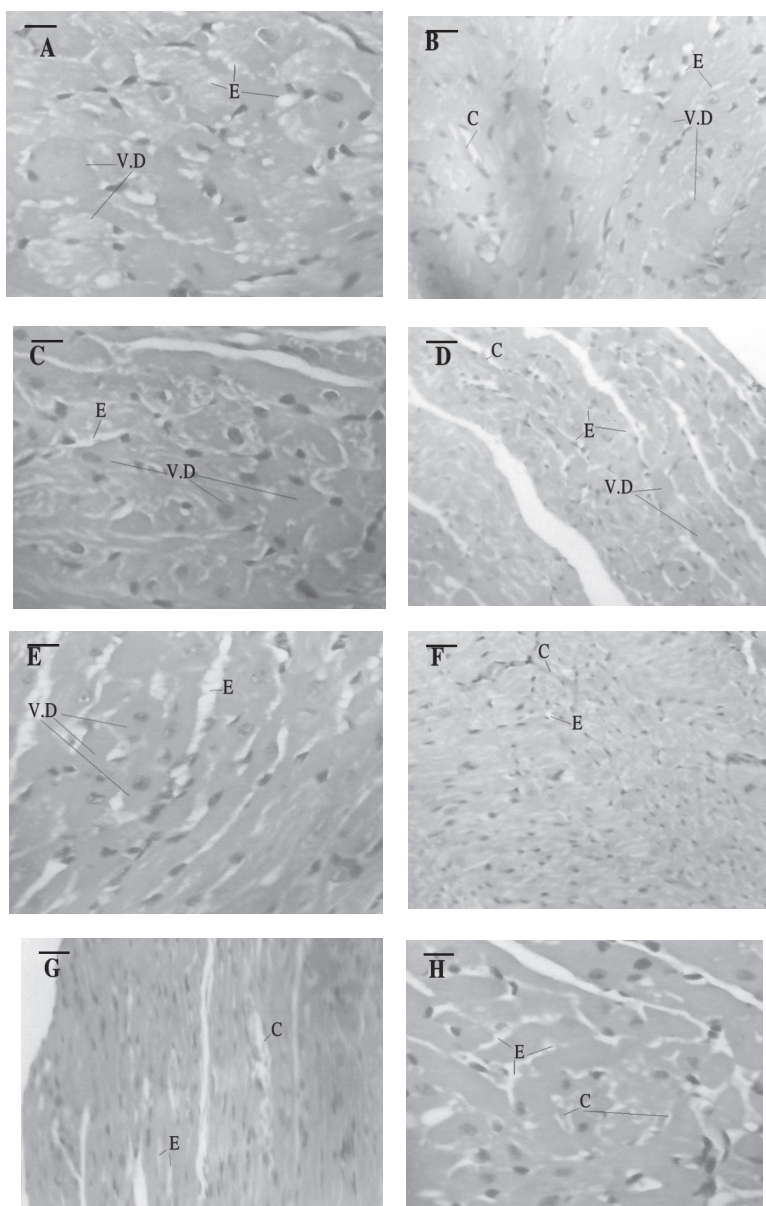


Fig. 3. Effect of methanolic plant extracts on doxorubicin (DOX)-induced alterations in cardiac histopathology. (A) CMC and DOX treatment shows severe vacuolar degeneration of the cardiac muscle (V.D) and interstitial edema (E); (B) treatment with *T. macropterus* extract and DOX shows focal V.D, mild E, and mild congestion (C); (C) treatment with *O. europaea* L. subsp. *africana* extract and DOX shows focal V.D and moderate E; (D) treatment with *T. aphylla* extract and DOX shows focal V.D, E, and C; (E) treatment with *C. coccineum* extract and DOX shows focal V.D and E; (F) treatment with *C. myxa* extract and DOX shows mild E and mild C; (G) treatment with *C. comosum* extract and DOX shows mild to moderate E and C; (H) treatment with *W. somnifera* extract and DOX shows severe interstitial E and C. Scale bar, 25 μ m.

tive effect of anthocyanins against DOX-induced toxicity in H9c2 cardiomyocytes in relation to their antioxidant activities. However, in the

present work, *C. coccineum* showed only fair *in vitro* radical scavenging and antioxidant activities. The results of the *in vivo* studies were in line with

those of the *in vitro* studies. The plant extract protected against DOX-induced GSH depletion, lipid peroxidation, and elevated activities of serum CK-MB and LDH, but did not alleviate the histopathological alterations induced by DOX.

In view of the reported antioxidant and hepatoprotective effects of *C. myxa* fruit extracts (Afzala *et al.*, 2007), fruits of this plant were included in the current study. The extract had excellent radical scavenging, reducing, and antioxidant activity, respectively. Antioxidant activity even exceeded that of ascorbic acid, and there was good protection against DOX-induced GSH depletion, MDA accumulation, and increase in serum marker activities, and partial protection against DOX-induced histological changes. These results are promising and warrant further investigations. Our findings are accord with those of Al-Awadi *et al.* (2001) and Afzala *et al.* (2007) on potent *in vitro* antioxidant activity, on enhancement of the antioxidant status in a rat model of colitis, and on a hepatoprotective effect. It remains to be seen whether the cardioprotective effect of *C. myxa* extract is mainly due to its antioxidative activity.

C. comosum is used in folk medicine to treat abdominal ailments; the stems and leaves are chewed for curing toothache (Ghazanfar, 1994) and as a hypoglycemic (El-Hawary and Kholief, 1990). Experimentally, aerial parts of *C. comosum* were shown to possess anti-inflammatory, anti-ulcer, and cytoprotective effects in rats (Liu *et al.*, 2001), as well as hypoglycemic activity (El-Hawary and Kholief, 1990). Evaluation of cytotoxic compounds such as catechin, dehydrodicathein A, kaempferol-3-*O*-rhamnopyranoside, quercitrin,

isoquercitrin, kaempferol-3-*O*-glucuronide, and mequilianin from *C. comosum* was reported by Badria *et al.* (2007). In the present study, the methanolic extract exhibited excellent radical scavenging and antioxidant activities. The cardiac markers were significantly improved, and there was mild protection against DOX-induced histological changes.

Many pharmacological studies have been carried out on the biological properties of *W. somnifera* (Mishra *et al.*, 2000), and cardioprotective activity has been reported (Mohanty *et al.*, 2004). *W. somnifera* positively influenced oxidative stress markers. The important medicinal properties of *W. somnifera* were attributed to the presence of a unique class of steroidal lactones called withanolide (Dhar *et al.*, 2006). In the current work, the plant showed excellent *in vitro* radical scavenging and antioxidant properties. Further, it significantly protected against DOX-mediated induction of GSH and MDA, as well as CK-MB and LDH, but it did not reverse the changes in histopathology.

It was obvious that antioxidant effects alone are not enough to provide cardioprotection. In conclusion, while the methanolic extracts of the seven plants exhibited significant radical scavenging and antioxidant activities, extracts from only two plants provided significant protection against DOX-induced cardiotoxicity, *C. comosum* > *C. myxa*.

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