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Thyme oil and thymol abrogate doxorubicin-induced nephrotoxicity and cardiotoxicity in Wistar rats *via* repression of oxidative stress and enhancement of antioxidant defense mechanisms

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Abstract: This study aimed to assess the preventive effects of thyme oil and thymol on doxorubicin (DOX)-induced renotoxicity, cardiotoxicity, and oxidative stress in Wistar rats. Thyme oil was subjected to GC-MS analysis, which indicated that thymol was the major constituent representing 33.896%. Rats intraperitoneally injected with DOX at a dose of 2 mg/kg b.w./one per week for 7 weeks were co-treated with thyme oil and its major constituent, thymol, at doses 250 and 100 mg/kg b.w./every other day, respectively, by oral gavage for the same period. Thyme oil and thymol markedly ameliorated the raised levels of serum urea, uric acid, and creatinine in DOX-administered rats. They also reduced the elevated activities of serum CK-MB and LDH. Thyme oil was more effective than thymol in decreasing the elevated serum creatinine level and serum CK-MB activity in DOX-administered rats, thereby reflecting its more potent effect on kidney and heart functions. Lipid peroxidation significantly decreased while GSH level and GST and GPx activities significantly increased in kidney and heart of DOX-administered rats treated with thyme oil and thymol. The DOX-induced perturbed kidney histological changes including congestion of glomerulus tuft, inflammatory cells infiltration, protein cast in lumina of the renal tubule, and thickening of the parietal layer of Bowman's capsule were remarkably ameliorated as a result of treatment with thyme oil and thymol; thyme oil was more effective. In addition, DOX-induced deleterious heart histological alterations, including intramuscular infiltration of inflammatory cells, focal necrosis of cardiac myocytes, and edema, were remarkably reduced by treatment with thyme oil and thymol. Thus, it can be concluded that DOX could induce marked toxicity in kidney and heart, and the treatment with thyme oil or thymol produced potential improvement of kidney and heart function and histological integrity *via* repression of oxidative stress and enhancement of antioxidant defense mechanisms.

Introduction

Doxorubicin (DOX), an anticancer agent from anthracycline antibiotic group, has a wide range of activity, and it has been used alone or in a mixture to cure various types of carcinomas (Jabłońska-Trypuć *et al*., 2017). Despite its common use for the treatment of many human cancers, DOX was found to induce significant cardiotoxicity (Kuznetsov *et al*., 2011) and nephrotoxicity (Mohan *et al*., 2010) that limit its clinical application. DOX-induced renal toxicity was reported to be mediated mainly *via* free radical increased production that eventually results in membrane lipid peroxidation (LPO) and cell damage (Ghibu *et al*., 2012; Refaie *et al*., 2016). In this regard, DOX has direct renal injurious effects as it preferentially accumulates in the kidney (Refaie *et al*., 2016) leading to tissue damage (Saad *et al*., 2001; Karaman *et al*., 2006). However, the cardiac side effects are also the main reason for the dose-limited administration of DOX (Carvalho *et al*., 2009). Several reasons have been elucidated to rationalize DOX-induced cardiotoxic effects, including exacerbated oxidative stress, mitochondrial damage, and cellular injuries (Ashrafi and Roshan, 2012). DOX causes an imbalance between the generation of free radicals and antioxidant defense, resulting in tissue injury (Essick and Sam, 2010) that is confirmed by elevated LPO and protein oxidation in respective organs (Karaman *et al*., 2006). Several publications have stated that an organism is protected from reactive oxygen species (ROS) oxidative damage by means of its antioxidant defense system, and the treatment with DOX modulates glutathione (GSH) and GSH-dependent

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antioxidant enzymes (Ashrafi and Roshan, 2012).

Many herbs have antioxidant potential by scavenging and counteracting free radicals or oxidants produced during various biochemical pathways (Rufino *et al*., 2009; 2010; Rahal *et al*., 2014). In addition, herbal medicine can enhance the response rate or chemosensitivity of drugs, reduce side-effects of chemotherapy, and prolong the survival time of patients with cancer diseases (Ben-Arye *et al*., 2017; Feng *et al*., 2018). *Thymus vulgaris*, "thyme", is a well-known herb with aromatic characteristics, and it is frequently used as a spice and herbal remedies since ancient times (Aljabeili *et al*., 2018). *T. vulgaris* essential oil contains a wide range of aromatic bioactive constituents (Marino *et al*., 1999; Božin *et al*., 2006; Gavaric *et al*., 2015). Thymol, one of the major components of thyme oil, has biological properties that include antibacterial, anti-inflammatory, and antioxidant activities (Bermejo *et al*., 2015; Aljabeili *et al*., 2018). As reported by Meeran and Prince (2012) and Meeran *et al*. (2015a; 2016), thymol has 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and reducing property in a dose-dependent manner. Furthermore, it was demonstrated that thymol has radioprotective (Archana *et al*., 2011), antioxidant (Yanishlieva *et al*., 1999), free radical scavenging (Fujisawa and Kadoma, 1992), anti-LPO (Beach and Giroux, 1992), and anti-inflammatory (Aeschbach *et al*., 1994) properties.

Therefore, this study was conducted to assess the reno-cardiac preventive effects of thyme oil and thymol on DOX-induced toxicity in Wistar rats by manifesting the effects on kidney and heart functions, oxidative stress and antioxidant defense system as well as histological integrity and architecture.

Materials and Methods

Experimental animals

Forty-eight male Wistar rats (120-150 g) used in the present investigation were purchased from the Animal House of the National Research Center (NRC), 33 El-Buhouth St. 12311 Dokki, Giza, Egypt. To prevent any intercurrent infections, animals were kept overseen under strict care for two weeks before the onset of the experiment. The animals were housed in polypropylene cages with well-aerated stainless-steel covers at a temperature of 25 ± 5 °C and a normal light-dark cycle. Animals had an adequate supply of water and enough balanced standard diet. All experimental procedures were in accordance with the guidelines and instructions of the Ethics Committee for the use and care of experimental animals, Faculty of Science, Beni-Suef University, Egypt (Ethical approval number: BSU/FS/2014/10). All efforts were done to refine the experiment and to reduce the pain, distress, and suffering of animals.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Chemical analysis of thyme oil was performed in the Central Laboratory of the Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Egypt, by using GC system 7890A/5975 Inert MS with Triple Axis Detector, Agilent Technologies, Germany. The constituting derivatives were identified by comparing their mass spectra with the spectra of derivatives in the Library Search Report [C:\Database\NIST11.L;C:\Database\demo.1].

Chemicals and drugs

DOX in the form of adriamycin hydrochloride was obtained from Pharmacia Italia (Milan, Italy). Serum uric acid and creatinine kinase MB (CK-MB) kits were obtained from Spinreact, Sant Esteve de Bas, Girona, Spain. Creatinine and urea kits were obtained from Biosystem S.A. (Spain). Lactate dehydrogenase (LDH) kit was obtained from Seppim S.A.S. Zone Industrielle, 61500 SEES, France. All other chemicals used in this study were of grade I and were commercially obtained.

Preparation of thyme oil dose

Thyme oil was obtained from Purity Factory, Abu-Radi Industrial Area, Food Sector, Alwasta, Beni-Suef, Egypt. Thyme oil dose was prepared by dissolving 250 mg in 5 mL of 1% carboxymethylcellulose (CMC) that was given to kilogram body weight (kg b.w.) of rats (Fachini-Queiroz *et al.*, 2012; Grespan *et al*., 2014) every other day for 7 weeks.

Preparation of thymol

Thymol was obtained from Riedel-De Haen AG Seelze-Hannover Company (Berlin, Hamburg), prepared by dissolving a dose of 100 mg/kg b.w. in 5 mL 1% CMC that was given to kg b.w. (Fachini-Queiroz *et al.*, 2012) every other day for 7 weeks.

Experimental design

Forty-eight male rats were dived into 4 groups (12 animals in each group) designed as follows: (1) Normal group: rats within this group received the same volume of the vehicle (5 mL 1% CMC)/kg b.w. every other day by oral gavage for 7 weeks. (2) DOX-administered control group: rats of this group were intraperitoneally injected with DOX at a dose of 2 mg/kg b.w. one time per week for 7 weeks. This group was also orally administered 5 mL 1% CMC/kg b.w. as the vehicle every other day for 7 weeks. (3) DOX-administered group treated with thyme oil: Rats of this group were intraperitoneally injected with DOX as those of the DOX-administered control group and were also orally treated with thyme oil at a dose of 250 mg (dissolved in 5 mL 1% CMC)/kg b.w. (Fachini-Queiroz *et al.*, 2012; Grespan *et al*., 2014) every other day by oral gavage for 7 weeks. (4) DOX-administered group treated with thymol: Rats of this group were intraperitoneally injected with DOX as those of the DOX-administered control group and were also orally treated with thymol at a dose of 100 mg (dissolved in 5 mL 1% CMC)/kg b.w. every other day (Fachini-Queiroz *et al.*, 2012) for 7 weeks.

Blood and tissue sampling

After treating the rats with the specific doses for 7 weeks, they were sacrificed under diethyl ether inhalation anesthesia. Blood was collected from the jugular vein of each animal in gel and clot activator tubes, left to clot at room temperature, and then centrifuged by using table-top centrifuge at 3000 rpm for 15 min. The sera were quickly aspirated with Pasteur pipette, fractioned into 4 portions for each animal, and kept at -30°C until different biochemical determinations. Kidney and heart tissues were quickly dissected out, weighed, and

homogenized in isotonic saline (0.9% NaCl) by using Teflon homogenizer (Glas-Col, Terre Haute, USA). The resultant homogenates were then centrifuged at 3000 rpm for 15 min, and the supernatants were aspirated and frozen at -30°C, until their use in the assay of biochemical parameters related to oxidative stress markers and antioxidant parameters.

Detection of kidney function biomarkers in serum

Urea, uric acid, and creatinine levels in serum were estimated according to the methods of Tabacco *et al.* (1979), Fossati *et al.* (1980), and Fabiny and Ertingshausen (1971), respectively.

Estimation of heart function parameters in serum

CK-MB and LDH activities in serum were assayed according to the methods of Young (1995) and Henderson and Moss (2001), respectively.

Estimation of liver lipid peroxidation (LPO) and antioxidant parameters

Kidney and heart LPO was estimated according to the method of Yagi (1987). GSH content was determined according to the method of Beutler *et al.* (1963). Glutathione-S-transferase (GST; EC 2.5.1.18) and glutathione peroxidase (GPx; EC 1.11.1.9) activities were assayed according to the methods of Mannervik and Guthenberg (1981) and Matkovics *et al.* (1997) respectively.

Histological examination

After decapitation by cervical dislocation and dissection, pieces of kidney and heart from all groups were rapidly dissected out from each animal, fixed in neutral buffered formalin (10%) for 48 h and then sent to Microanalysis and Environmental Research Center, Molecular Biology Unit, Faculty of Science, Beni-Suef University, Egypt for processing and staining with hematoxylin and eosin (H & E) (Banchroft *et al.*, 1996) and examined for detection of lesions.

Statistical analysis

The obtained data were represented as mean ± standard error mean (SEM). They were analyzed using the one-way analysis of variance (ANOVA) followed by LSD test (PC-STAT, University of Georgia, 1985) to compare different groups with each other (Rao *et al.*, 1985) at $p < 0.05$ and $p < 0.01$. F-probabilities obtained by one-way ANOVA expressed the general effect between groups at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results

GC-MS analysis of thyme oil extract

The GC-MS analysis (Tab. 1 and Fig. 1) indicated the presence of multiple phytochemicals. The main constituents and groups which have concentration more than 1% of total include p-cymene, linalool, endo-borneol, terpinen-4-ol, thymol, carvacrol, and caryophyllene oxide. Thymol and p-cymene have the highest percentage of the total.

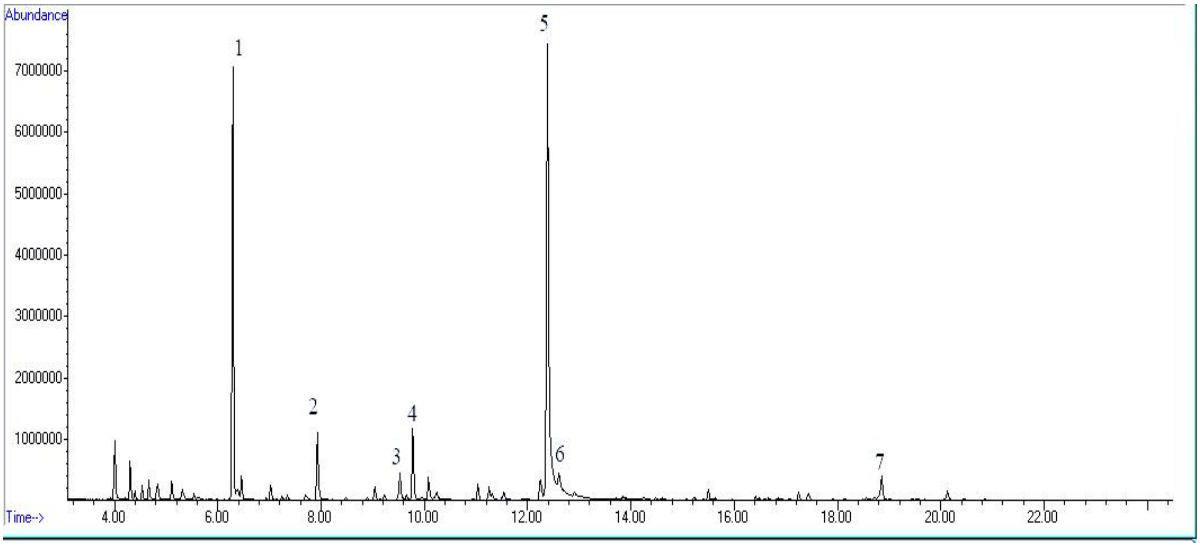

FIGURE 1. GC-MS chromatogram of thyme oil.

TABLE 1

Chemical composition of thyme oil as detected by GC-MS analysis

Number	Retention time	Compound (from Central Library Search Report)	Area % (higher than 1%)
1	6.292	p-cymene	20.48577
2	7.932	linalool	3.596444
3	9.530	endo-borneol	1.776999
4	9.781	terpinen-4-ol	3.714859
5	12.386	thymol	33.89638
6	12.608	carvacrol	4.653145
7	18.856	caryophyllene oxide	2.237118

Effect on kidney function parameters in serum

DOX administration for 7 weeks produced a highly significant ($p < 0.01$; LSD) increase in uric acid and creatinine levels; the recorded percentage increases were +123.08 and +16.13, respectively, as compared to normal. The serum urea level, on the other hand, significantly ($p < 0.05$; LSD) increased in DOX-administered rats recording percentage increase of +20.96 as compared to the normal group.

The treatment in DOX-administered rats with thyme oil produced a highly significant decrease ($p < 0.01$; LSD) of serum uric acid level recording percentage change of -38.62 and a significant amendment ($p < 0.05$; LSD) in serum creatinine level recording percentage change of -11.11 as compared to DOX-administered control. In contrast, serum urea level non-significantly decreased ($p > 0.05$; LSD) as a result of treatment of DOX-administered rats with thyme oil; the recorded percentage change was -13.43 as compared to DOX-administered control. Thymol supplementation to DOX-administered rats induced a highly significant decrease ($p < 0.01$; LSD) in urea and uric acid levels recording percentage changes of -22.94 and -40.00 respectively, while it produced a non-significant effect ($p > 0.05$; LSD) on serum creatinine concentration recording percentage change of -4.17 as compared to DOX-administered control (Tab. 2).

Effect on heart function biomarkers in serum

DOX injection for 7 weeks stimulated a significant ($p < 0.05$; LSD) and highly significant ($p < 0.01$; LSD) increase in serum CK-MB and LDH activities recording percentage changes of +52.00 and +63.24 respectively as compared to the normal group. DOX-administered rats treated with thyme oil produced a highly significant ($p < 0.01$; LSD) and a significant ($p < 0.05$; LSD) ameliorative effect on CK-MB and LDH activities recording percentage changes of -38.42 and -20.85 respectively as compared to DOX-administered control. On the other hand, the treatment of DOX-administered rats with thymol induced a significant ($p < 0.05$; LSD) and highly significant ($p < 0.01$; LSD) effect on LDH and CK-MB activities respectively recording percentage changes of -31.89 and -28.60 respectively as compared to DOX-administered control (Tab. 3).

Effect on kidney and heart oxidative stress and antioxidant defense parameters

DOX injection for 7 weeks evoked a highly significant ($p < 0.01$; LSD) increase in LPO in the kidney and recorded a percentage change of +62.27 as compared to the normal group. It also produced a highly significant ($p < 0.01$; LSD) decrease in kidney GSH content and GST and GPx activities; the recorded percentage decreases were -36.16, -29.53, and -19.73%, respectively. The treatment of DOX-administered rats with thyme oil and thymol produced a significant ($p < 0.05$; LSD) improvement of the elevated LPO in kidney recording percentage changes of -27.79 and -22.81 respectively as compared to DOX-administered control. The treatment of DOX-administered rats with thyme oil and thymol produced a significant ($p < 0.05$; LSD) increase in the lowered kidney GSH content and GPx activity and a highly significant ($p < 0.01$; LSD) increase in kidney GST activity as compared to DOX-administered control (Tab. 4).

Similar to the kidney, DOX administration for 7 weeks induced a highly significant ($p < 0.01$; LSD) increase in LPO in heart recording percentage increase of +38.17, while it produced a highly significant ($p < 0.01$; LSD) decrease in heart GSH content, GST activity, and GPx activity; the recorded percentage changes were -32.03, -37.94 and -13.05% respectively as compared to normal group. The treatment of DOX-administered rats with thyme oil and thymol produce a significant improvement ($p < 0.05$; LSD) of the elevated LPO in heart recording percentage decreases of -18.49 and -33.28 respectively as compared to DOX-administered control. Moreover, the treatment of DOX-administered rats with thyme oil produced a significant enhancement ($p < 0.05$; LSD) in lowered heart GSH content, GST activity, and GPx activity; the recorded percentage changes were +26.27, +18.32 and +9.85% respectively as compared to DOX-administered control. While thymol treatment produced a significant ($p < 0.05$; LSD) increase in lowered heart GSH content and GST activity reaching near their normal levels, it produced a detectable but non-significant ($p > 0.05$; LSD) increase in heart GPx activity recording percentage change of +5.24 as compared to DOX-administered control (Tab. 5).

TABLE 2

Effects of thyme oil and thymol administration on kidney function parameters in serum of DOX-administered rats

Parameters / Groups	Urea (mg/L)	% change	Uric acid (mg/dL)	% change	Creatinine (mg/dL)	% change
Normal	31.83 ± 2.65[b]	-	1.30 ± 0.13[b]	-	0.62 ± 0.04[c]	-
DOX-administered control	38.50 ± 1.12[a]	+20.96	2.90 ± 0.26[a]	+123.08	0.72 ± 0.01[a]	+16.13
DOX-administered group treated with thyme oil	33.33 ± 1.02[ab]	-13.43	1.78 ± 0.21[b]	-38.62	0.64 ± 0.02[bc]	-11.11
DOX-administered group treated with thymol	29.67 ± 2.91[b]	-22.94	1.73 ± 0.06[b]	-40.00	0.69 ± 0.01[ab]	-4.17
F-probability	$p < 0.05$		$p < 0.001$		$p < 0.05$	
LSD at the 5% level	6.22		0.53		0.07	
LSD at the 1% level	8.48		0.72		0.09	

Data are expressed as mean ± standard error mean (SEM).

Number of animals in each group is six.

For each parameter, means, which share the same superscript symbol (s), are not significantly different.

Percentage changes were calculated by comparing DOX-administered control with normal and DOX-administered groups treated with thyme oil and thymol with DOX-administered control.

TABLE 3

Effects of thyme oil and thymol administration on heart function parameters in serum of DOX-administered rats

Parameters / Groups	CK-MB (U/L)	% change	LDH (U/L)	% change
Normal	62.50 ± 2.59[b]	-	1721.67 ± 104.75[c]	-
DOX-administered control	95.00 ± 15.23[a]	+52.00	2810.50 ± 18.13[a]	+63.24
DOX-administered group treated with thyme oil	58.50 ± 4.02[b]	-38.42	2224.50 ± 173.10[b]	-20.85
DOX-administered group treated with thymol	64.70 ± 5.00[b]	-31.89	2006.83 ± 247.73[bc]	-28.60
F-probability	$p < 0.05$		$p < 0.001$	
LSD at the 5% level	24.67		472.55	
LSD at the 1% level	33.65		644.48	

Data are expressed as mean ± standard error mean (SEM).

Number of animals in each group is six.

For each parameter, means, which share the same superscript symbol(s), are not significantly different.

Percentage changes were calculated by comparing DOX-administered control with normal and DOX-administered groups treated with thyme oil and thymol with DOX-administered control.

TABLE 4

Effects of thyme oil and thymol administration on LPO, GSH content and GST and GPx activities in kidney of DOX-administered rats

Parameters / Groups	LPO (nmole MDA/100 mg tissue/h)	% change	GSH content (nmole/100 mg tissue)	% change	GST activity (mU/100 mg tissue)	% change	GPx activity (U/100 mg tissue)	% Change
Normal	26.48 ± 0.87^{b}	-	38.58 ± 3.01^{a}	-	202.25 ± 11.78^{ab}	-	149.71 ± 3.36^{a}	-
DOX-administered control	42.97 ± 4.63^{a}	+62.27	24.63 ± 1.58^{b}	-36.16	142.53 ± 5.36^{c}	-29.53	120.17 ± 5.60^{b}	-19.73
DOX-administered group treated with thyme oil	31.03 ± 3.57^{b}	-27.79	34.97 ± 3.06^{a}	+41.98	205.21 ± 9.48^{a}	+43.98	136.81 ± 6.27^{a}	+13.85
DOX-administered group treated with thymol	33.17 ± 2.13^{b}	-22.81	35.25 ± 4.65^{a}	+43.12	179.58 ± 5.87^{b}	+25.99	137.36 ± 5.19^{a}	+14.30
F-probability	$p < 0.05$		$p < 0.05$		$p < 0.001$		$p < 0.01$	
LSD at the 5% level	9.27		9.62		25.20		15.39	
LSD at the 1% level	12.65		13.12		34.37		20.98	

Data are expressed as mean ± standard error mean (SEM).

Number of animals in each group is six.

For each parameter, means, which share the same superscript symbol(s), are not significantly different.

Percentage changes were calculated by comparing DOX-administered control with normal and DOX-administered groups treated with thyme oil and thymol with DOX-administered control.

TABLE 5

Effects of thyme oil and thymol administration on LPO, GSH content and GST and GPx activities in heart of DOX-administered rats

Parameters / Groups	LPO (nmole MDA/100 mg tissue/h)	% change	GSH content (nmole/100 mg tissue)	% change	GST activity (mU/100 mg tissue)	% change	GPx activity (U/100 mg tissue)	% change
Normal	12.68 ± 1.23^{b}	-	29.07 ± 1.40^{a}	-	75.55 ± 3.38^{a}	-	124.99 ± 1.86^{a}	-
DOX-administered control	17.52 ± 0.62^{a}	+38.17	19.76 ± 1.27^{b}	-32.03	46.89 ± 2.97^{c}	-37.94	108.68 ± 4.01^{c}	-13.05
DOX-administered group treated with thyme oil	14.28 ± 1.36^{b}	-18.49	24.95 ± 1.78^{a}	+26.27	55.48 ± 2.73^{b}	+18.32	119.39 ± 2.26^{ab}	+9.85
DOX-administered group treated with thymol	11.69 ± 0.96^{b}	-33.28	24.48 ± 1.84^{a}	+23.89	76.21 ± 2.12^{a}	+62.53	114.37 ± 5.10^{bc}	+5.24
F-probability	$p < 0.01$		$p < 0.01$		$p < 0.001$		$p < 0.05$	
LSD at the 5% level	3.18		4.69		8.37		10.49	
LSD at the 1% level	4.34		6.40		11.41		14.31	

Data are expressed as mean ± standard error mean (SEM).

Number of animals in each group is six.

For each parameter, means, which share the same superscript symbol(s), are not significantly different.

Percentage changes were calculated by comparing DOX-administered control with normal and DOX-administered groups treated with thyme oil and thymol with DOX-administered control.

Kidney histological changes

Light microscopic investigation of the kidney of the normal group revealed normal glomeruli as well as proximal and distal convoluted tubules (Fig. 2). As a result of DOX administration, the kidney exhibited several deleterious histological changes and lesions (Fig. 3). The kidney of DOX-administered rats showed congestion of glomerulus tuft and inflammatory cells infiltration (Fig. 3(a)), nephritis, interstitial inflammatory cells infiltration and protein cast in the lumina of renal tubules (Fig. 3(b)), interstitial strands of fibroblasts proliferation (Fig. 3(c)), and congestion of glomerular tuft together with thickening of Bowman's capsule parietal layer (Fig. 3(d)).

The treatment of DOX-administered rats with thyme oil (Fig. 4) and thymol (Fig. 5) resulted in a marked amendment of the DOX-induced kidney lesions. The kidney of DOX-administered rats treated with thyme oil revealed improvement and nearly normal structure of the renal tissue; glomerulus as well as proximal and distal tubules (Fig. 4). The kidney of DOX-administered rats treated with thymol showed slight congestion of capillaries in glomerulus tuft (Fig. 5).

Heart histological changes

Light microscopic examination of the heart of the normal group revealed normal histological architecture and normal cardiac myocytes (Fig. 6).

The DOX administration produced many heart histological deleterious changes (Fig. 7). The heart of DOX-administered rats showed interstitial inflammatory cells infiltration (Fig. 7(a)), focal necrosis of cardiac myocytes associated with inflammatory leucocytic infiltration (Fig. 7(b)), as well as focal necrosis of cardiac myocytes associated with both inflammatory cells infiltration and edema (Fig. 7(c)).

The treatment of DOX-administered rats with thyme oil (Fig. 8) and thymol (Fig. 9) produced a marked alleviation of DOX-induced histological changes. The heart of DOX-administered rats treated with thyme oil and thymol revealed mild myocarditis and inflammatory cells infiltration between cardiac myocytes.

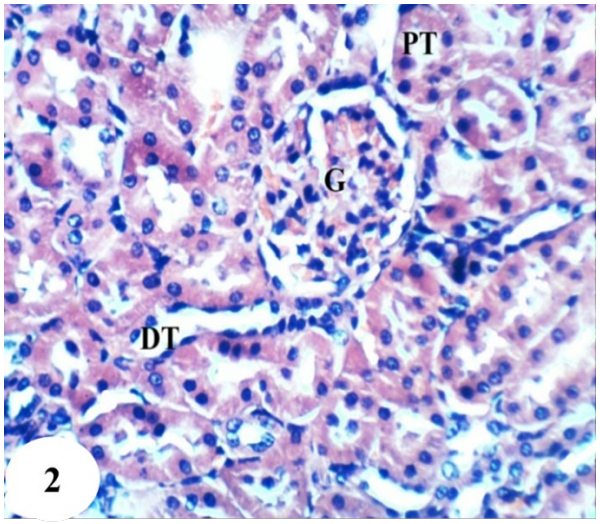

FIGURE 2. Photomicrograph of normal kidney section depicting normal glomeruli (G), proximal tubules (PT), and distal tubules (DT). (H & E; 400X).

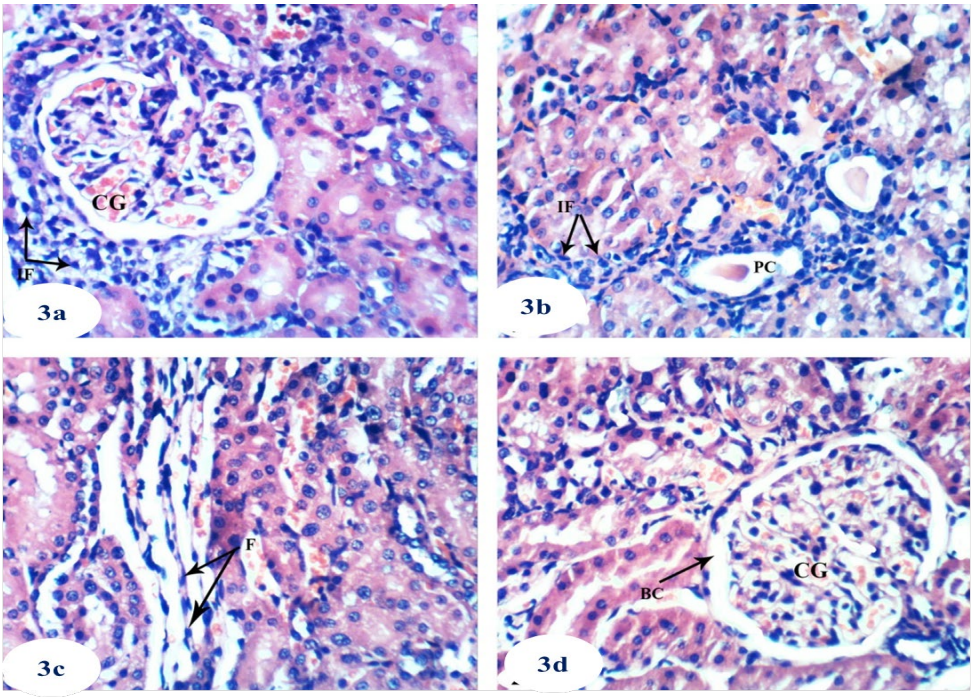

FIGURE 3. Photomicrographs of kidney sections of DOX-administered rats showing **(3a)** congestion of glomerulus tuft (CG), inflammatory cells infiltration (IF); **(3b)** nephritis; interstitial inflammatory cells infiltration (IF), and protein cast (PC) in the lumens of renal tubules; **(3c)** interstitial strands of fibroblasts proliferation (F); and **(3d)** congestion of glomerular tuft (CG) together with thickening in parietal layer of Bowman`s capsule (BC). (H & E; 400X).

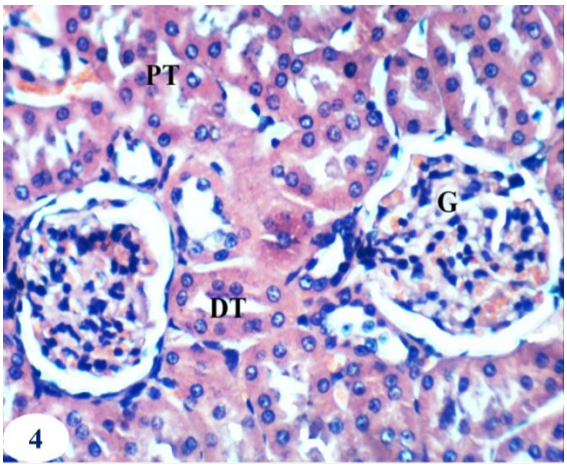

FIGURE 4. Photomicrograph of kidney section of DOX-administered rat treated with thyme oil showing improvement and nearly normal histological structure of the renal tissue depicting glomeruli (G) proximal tubules (PT) and distal tubules (DT). (H & E; 400X).

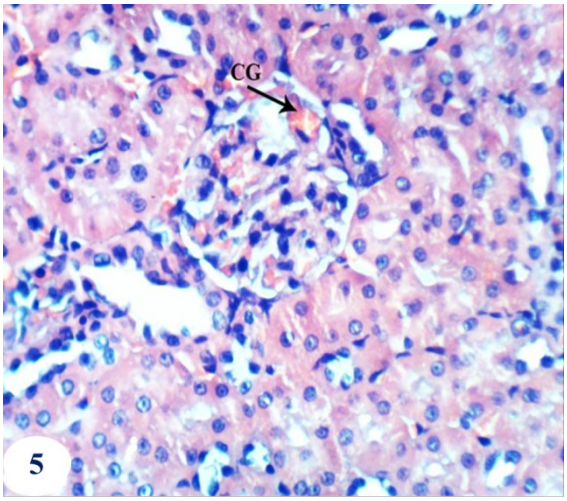

FIGURE 5. Photomicrograph of kidney section of DOX-administered rat treated with thymol showing slight congestion of glomerulus tuft (CG). (H & E; 400X).

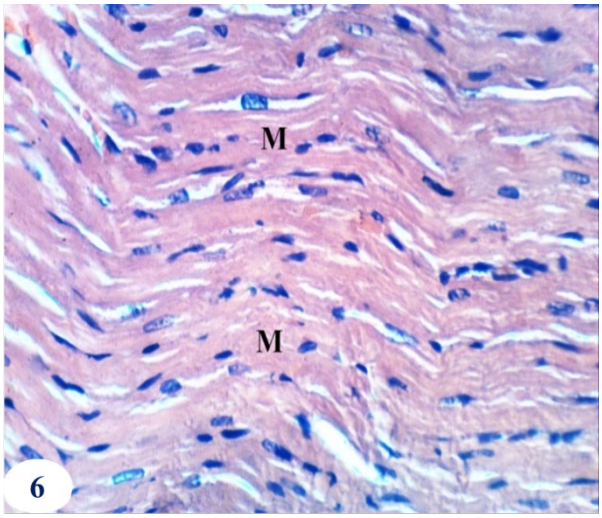

FIGURE 6. Photomicrograph of normal heart section showing normal histological architecture and normal cardiac myocytes (M). (H & E; 400X).

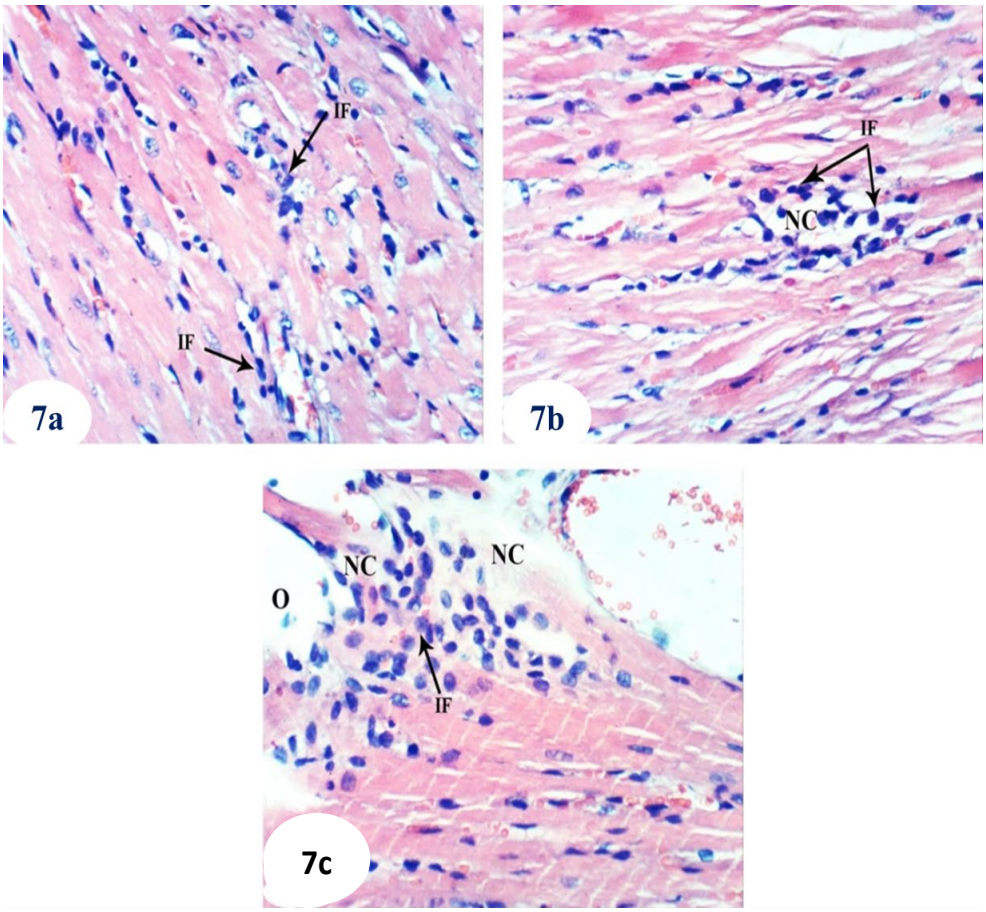

FIGURE 7. Photomicrographs of heart sections of DOX-administered rats showing **(7a)** intramuscular inflammatory cells infiltration (IF); **(7b)** focal necrosis of cardiomyocytes (NC) together with inflammatory cells infiltration (IF); **(7c)** focal necrosis of cardiomyocytes (NC) accompanied with inflammatory cells infiltration (IF) and edema (O). (H & E; 400X).

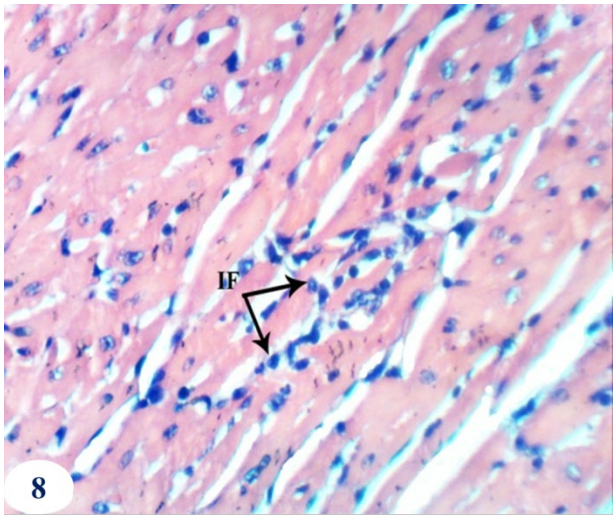

FIGURE 8. Photomicrograph of heart section of DOX-administered rat treated with thyme oil showing mild myocarditis and intramuscular inflammatory cells infiltration (IF). (H & E; 400X).

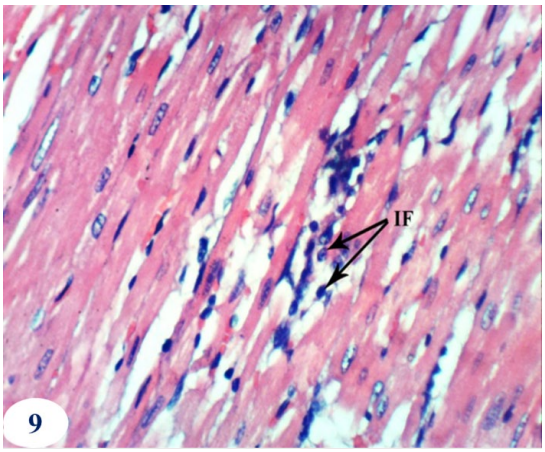

FIGURE 9. Photomicrograph of heart section of DOX-administered rat treated with thymol showing mild myocarditis and intramuscular inflammatory cells infiltration (IF). (H & E; 400X).

Discussion

DOX is widely used to treat a variety of cancer diseases. Its administration may lead to side effects and organ toxicity, including nephrotoxicity and cardiotoxicity that may be mediated *via* DOX-induced oxidative stress (Zheng *et al*., 2011; Rashid *et al*., 2013).

The present current study demonstrated that the effect of intraperitoneal injection of DOX at a dose level of 2 mg/kg b.w. one day/week for 7 weeks induced nephrotoxicity manifested biochemically by a significant increase in serum urea, uric acid, and creatinine levels. The elevation in serum urea, uric acid, and creatinine levels provides evidence that DOX impairs kidney function and reduces the ability of the kidney to remove toxic metabolic substances. These results are in accordance with Injac *et al*. (2008), Ahmed *et al*. (2013), and Ahmed *et al*. (2017), who showed elevated levels of serum urea, uric acid, and creatinine in DOX-administered rats. Histopathological examination of kidney sections of DOX-administered rats, in the present study, supported the previous biochemical results. As a result of DOX injection, the kidney exhibited several deleterious histological changes and lesions, including congestion of glomerulus tuft, nephritis, interstitial inflammatory leucocytic infiltration, protein cast in the renal tubule lumina, interstitial strands of fibroblasts' proliferation, and thickening of Bowman's capsule parietal layer. These histological changes are in concurrence with other publications (Hahn *et al*., 2004; Refaie *et al*., 2016; Ahmed *et al*., 2017; Ahmed *et al*., 2019). In the same regard, El-Moselhy and El-Sheikh (2014) attributed DOX-induced nephrotoxicity to the finding that DOX-administration increased capillary permeability and glomerular atrophy. In our opinion, the histological perturbances in the kidney after DOX injection may be due to the excessive production of free radicals, increase in the production of ROS, and attenuation of the antioxidant defense. The results of the present study support this attribution since the DOX administration to rats elevation in renal LPO and a significant decrease in renal GSH content as well as GPx and GST enzyme activities.

In the present study, the DOX administration for 7 weeks, induced impairment in heart function, which was marked biochemically by a significant rise in serum CK-MB and LDH activities. These changes are in concordance with El-Sayed *et al*. (2016) study, which showed a significant elevation in serum CK-MB and LDH activities reflecting DOX-induced cardiac damage. Histopathological results provided supportive evidence for the alterations in activities of serum enzymes related to heart function. In the present study, the heart of DOX-administered rats exhibited interstitial leucocytic cells infiltration, focal necrosis of cardiomyocytes, and edema. These histological results are in agreement with those obtained by Ahmed *et al*. (2017; 2019), who depicted necrotic cells and inflammatory cells infiltration in the heart of DOX-administered rats. These deleterious changes in heart function and histological integrity may be secondary to the stimulation in the heart oxidative stress and decrease in GSH and enzymatic antioxidants (GPx and GST), as evidenced in the present investigation.

DOX causes deterioration in the balance between ROS and antioxidants. This perturbance in oxidant-antioxidant systems, in turn, results in LPO and protein oxidation and tissue injury (Karaman *et al*, 2006). The deleterious biochemical and histological alterations of the current study were concomitant with a marked elevation of kidney and heart LPO and a significant depletion in non-enzymatic antioxidants (GSH) content and antioxidant enzymes (GPx and GST). These results are in currencies with Ahmed *et al*. (2017; 2019), who demonstrated elevated LPO, depleted GSH content, and suppressed antioxidant enzyme activities in both kidney and heart of DOX-administered rats. In the same way, Liu *et al*. (2007) found that administration of DOX caused decreases in kidney GSH and GST in rats when compared to the normal control group. According to these publications, DOX-induced severe nephrotic syndrome with massive albuminuria, proteinuria, hyperlipidemia, hypoalbuminemia, and hypoproteinemia was associated with a marked suppression in the kidney antioxidant defense and activation of oxidative stress. These later changes were evidenced in the present study by the increase in kidney LPO and the decrease of kidney GSH content and GPx activity in DOX-administered rats. In addition, Saad *et al*. (2001) found a marked increase in LPO levels and depletion of GSH contents

in the heart, kidney, and liver in association with impairments in serum functional biochemical parameters related to these organs and histological architecture and integrity in DOX-administered rats. Thus, the results of the present study and past publications lead us to suggest that the oxidative stress stimulated and the attenuation of the antioxidant defense system play an important role in DOX-induced impairments in kidney and heart functions and histological integrities.

In the current work, the treatment of DOX-administered rats with thyme oil and thymol improved the kidney function profile by decreasing the urea and creatinine levels; these results are in concurrence with previous publications (Jovanović *et al*., 1996; Mansour *et al*., 1999). Also, the level of uric acid in the present study was found to be ameliorated after the treatment with thyme oil and thymol, and this effect is in concordance with Meeran *et al*. (2014). It is very related to mention here that these improvements in kidney function parameters in serum were concomitant with an amendment in the kidney histological integrity as well as with the suppression of kidney LPO and enhancement of the kidney antioxidant defenses. Thus, it can be suggested that the improvement in kidney function and histological integrity as a result of treatment of DOX-administered rats with thyme oil and thymol may be attributed, at least in part, to the suppression of kidney oxidative stress and enhancement of the kidney antioxidant defense mechanisms.

The present study revealed the ameliorative efficacy of thyme oil and thymol treatment on the elevated CK-MB and LDH activities in DOX-administered rats. These alleviations are in a good concurrence with Guesmi *et al*. (2018) who demonstrated that oily fraction of *Thymus algeriensis* potentially produced potent cardioprotective effects against hydrogen peroxide-induced cardiotoxicity in rats and with El-Sayed *et al*. (2016) who stated that thymol successfully prevented DOX-induced cardiotoxicity by repression of oxidative stress, inflammation, and apoptosis. A study of Meeran *et al*. (2015b) showed oral administration of thymol prevents myocardial membrane destabilization by suppressing myocardial oxidative stress, decreasing LPO products in heart, improving antioxidant enzyme activities, and thereby reducing leakage out of the cardiac cytoplasmic enzymes such as CK-MB, AST, and LDH into the circulation.

In the present investigation, the treatment of DOX-administered rats with either thyme oil or thymol markedly improved the DOX-induced histological lesions in the kidney and heart. The kidney of DOX-administered rats treated with thyme oil completely normalized the kidney architecture while the kidney of DOX-administered rats treated with thymol still depicted mild congestion in the glomerular tuft. Otherwise, the heart of DOX-administered rats treated with thyme oil or thymol exhibited improvement in the heart histological architecture but still depicted mild myocarditis and slight infiltration of inflammatory cells. These alleviations are in concurrence with many previous reports. Guesmi *et al*. (2018) reported that the oily fraction of *T. algeriensis* protected against H_2O_2-induced heart histopathological alterations. Abd El Kader and Mohammed (2012) found that thyme extract treatment resulted in a marked improvement in most proximal and distal tubules and glomeruli of paracetamol-administered rats. El-Sayed *et al*. (2015) noticed that the pre-treatment of cisplatin-administered rats with thymol obviously mitigated cisplatin-induced kidney histopathological changes. El-Sayed *et al*. (2016) reported that thymol prevents doxorubicin-induced cardiotoxicity and deleterious heart histological alterations. Those authors attributed these nephro-cardio-preventive effects to abrogation effects of thymol on oxidative stress, inflammation, and apoptosis in paracetamol- and DOX-administered rats.

In association with the ameliorative effects of thyme oil or thymol on biochemical alterations related to kidney and heart functions in serum as well as on kidney and heart histological alterations in DOX-administered rats, the oxidative stress represented by LPO and antioxidant defense system represented by GSH content together with GPx and GST activities in both organs were remarkably improved. These amendments are in agreement with El-Sayed *et al*. (2015; 2016), who reported that thymol significantly improved the LPO, GSH content, and antioxidant enzyme activities in the kidney of cisplatin-administered rats and in the heart of DOX-administered rats. Guesmi *et al*. (2018) also elucidated that the oily fraction of *T. algeriensis* significantly lowered H_2O_2-induced heart protein oxidation and LPO and enhanced heart antioxidant defense enzymes. Furthermore, Tsai *et al*. (2011) found that the *T. vulgaris* essential oil, which has plentiful thymol, exhibited the most effective antioxidant potencies when compared with the essential oils of other four plants commonly used in Saudi Arabia.

In the present study, the thyme oil had more potent effect than thymol on improving the elevated level of the most specific marker of kidney function-creatinine-and the elevated serum activity of the most specific enzyme biomarker of heart function-CK-MB-as well as on kidney histological changes in DOX-administered rats due to presence phytochemicals other than thymol in thyme oil. The GC-MS analysis of thyme oil, in the present study, indicated the presence of p-cymene, linalool, endo-borneol, terpinen-4-ol, thymol, carvacrol, and caryophyllene oxide. Most of these components have antioxidant properties.

In conclusion, the thyme oil and its major component, thymol, have a preventive effect against the DOX-induced deleterious changes in kidney and heart function and histological integrity *via* the suppressive effect on oxidative stress and the enhancement effect on antioxidant defense system in both organs. Moreover, thyme oil was more effective than thymol on kidney and heart functions due to the presence of antioxidant phytochemicals rather than thymol.

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Conflicts of Interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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