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. GPxs 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
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, 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, 42 male Wistar rats (120-150 g) were used in the present study. To prevent any intercurrent infections, National Research Center (NRC), 33 El-Buhouth St. 12311 Dokki, Giza, Egypt. To prevent any intercurrent infections, rats were used in the present study. To prevent any intercurrent infections, rats were used in the present study.

Materials and Methods

Experimental design

Forty-eight male rats were divided 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.
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 urea 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) compared to DOX-administered control. While thymol treatment produced a significant ($p < 0.05$; LSD) increase in lowered heart GSH content and GST activity, and GPx activities; the recorded percentage decreases were -32.03, -37.94 and -13.05%, respectively as compared to normal group. The treatment of DOX-administered rats with thyme oil produced a significant enhancement ($p < 0.05$; LSD) improvement of the elevated LPO in heart 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 lower 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) decrease in kidney GSH content and GST activity as compared to normal group. It also produced a highly significant ($p < 0.01$; LSD) increase in lowered kidney GSH content and GPx activity as a highly significant ($p < 0.01$; LSD) increase in kidney GST activity as compared to DOX-administered control (Tab. 4).

Effect on heart function biomarkers in serum
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) decrease in kidney GST activity 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 -32.03, -37.94 and -13.05% respectively as compared to normal group. The treatment of DOX-administered rats with thyme oil and thymol produced a significant ($p < 0.05$; LSD) decrease in LPO heart recording percentage decreases of -18.49 and -33.28 respectively as compared to the normal group. It also produced a highly significant ($p < 0.01$; LSD) decrease in heart GSH content, GST activity, and GPx activity; 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) decrease in heart GSH content and GST activity as compared to normal group. It also 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).

Effect on kidney and heart oxidative stress and antioxidant defense parameters

**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               |
### 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 ± 2.65b | -         | 1.30 ± 0.13b      | -        | 0.62 ± 0.04c        | -        |
|            | DOX-administered control      | 38.50 ± 1.12b | +20.96    | 2.90 ± 0.26a      | +123.08  | 0.72 ± 0.01b        | +16.13   |
|            | DOX-administered group treated with thyme oil | 33.33 ± 1.02ab | -13.43   | 1.78 ± 0.21b      | -38.62   | 0.64 ± 0.02bc       | -11.11   |
|            | DOX-administered group treated with thymol | 29.67 ± 2.91b | -22.94   | 1.73 ± 0.06b      | -40.00   | 0.69 ± 0.01ab       | -4.17    |

F-probability:  
LSD at the 5% level:  
LSD at the 1% level:

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 ± 2.50b | -         | 1721.67 ± 104.75c | -        |
|            | DOX-administered control      | 95.00 ± 15.23a | +52.00   | 2810.50 ± 18.13a | +63.24   |
|            | DOX-administered group treated with thyme oil | 58.50 ± 4.02b | -38.42   | 2224.50 ± 173.10b | -20.85   |
|            | DOX-administered group treated with thymol | 64.70 ± 5.00b | -31.89   | 2006.83 ± 247.73bc | -28.60   |

F-probability:  
LSD at the 5% level:  
LSD at the 1% level:

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<sup>a</sup>       | -        | 38.58 ± 3.01<sup>b</sup>        | -        | 202.25 ± 11.78<sup>a</sup>     | -        | 149.71 ± 3.36<sup>a</sup>     | -        |
|             | DOX-administered control   | 42.97 ± 4.63<sup>a</sup>       | +62.27   | 24.63 ± 1.58<sup>a</sup>        | -36.16   | 142.53 ± 5.36<sup>a</sup>      | -29.53   | 120.17 ± 5.60<sup>a</sup>     | -19.73   |
|             | DOX-administered group treated with thyme oil | 31.03 ± 3.57<sup>a</sup> | -27.79  | 34.97 ± 3.06<sup>a</sup> | +41.98  | 205.21 ± 9.48<sup>a</sup> | +43.98  | 136.81 ± 6.27<sup>a</sup> | +13.85 |
|             | DOX-administered group treated with thymol | 33.17 ± 2.13<sup>a</sup> | -22.81  | 35.25 ± 4.65<sup>a</sup> | +43.12  | 179.58 ± 5.87<sup>a</sup> | +25.99  | 137.36 ± 5.19<sup>a</sup> | +14.30 |

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<sup>a</sup>       | -        | 29.07 ± 1.40<sup>a</sup>        | -        | 75.55 ± 3.38<sup>a</sup>      | -        | 124.99 ± 1.86<sup>a</sup>     | -        |
|             | DOX-administered control   | 17.52 ± 0.62<sup>a</sup>       | +38.17   | 19.76 ± 1.27<sup>a</sup>        | -32.03   | 46.89 ± 2.97<sup>a</sup>      | -37.94   | 108.68 ± 4.01<sup>a</sup>     | -13.05   |
|             | DOX-administered group treated with thyme oil | 14.28 ± 1.36<sup>a</sup> | -18.49  | 24.95 ± 1.78<sup>a</sup> | +26.27  | 55.48 ± 2.73<sup>a</sup> | +18.32  | 119.39 ± 2.26<sup>a</sup> | +9.85 |
|             | DOX-administered group treated with thymol | 11.69 ± 0.96<sup>a</sup> | -33.28  | 24.48 ± 1.84<sup>a</sup> | +23.89  | 76.21 ± 2.12<sup>a</sup> | +62.53  | 114.37 ± 5.10<sup>a</sup> | +5.24 |

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).

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).
mediated including nephrotoxicity and cardiotoxicity that may be administration may lead to side effects and organ toxicity, DOX is widely used to treat a variety of cancer diseases. Its

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 leukocytic 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 leukocytic 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, as evidenced in the present investigation.

DOX causes deterioration in the balance between ROS and antioxidants. This perturbation 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.

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).
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 relevant 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 carophyllene 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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