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Aspernolide F, as a new cardioprotective butyrolactone against doxorubicin-induced cardiotoxicity

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ABSTRACT

Endophytic fungi have known as a promising source of secondary metabolites. γ-Butyrolactones are a class of metabolites reported from *Aspergillus* genus, which attracted much attention for their bioactivities. This study aimed to assess the potential cardioprotective effects of aspernolide F (AF) separated from the endophytic fungus *A. terreus* against doxorubicin (DOX)-induced cardiotoxic effects in rats. Animals were treated with two different doses of AF for 10 days prior to DOX injection. Electrocardiographic (ECG), biochemical, histopathological and immunohistochemical analyses were performed. Results have shown that AF effectively protected against DOX-induced cardiac damage as AF counteracted DOX-induced ECG abnormalities and attenuated serum markers of cardiotoxicity (creatine kinase-MB, lactate dehydrogenase, troponin I, and troponin T). Histopathological examination of cardiac tissue revealed a remarkable improvement in DOX-induced lesions. In addition, AF ameliorated DOX-induced oxidative damage and increased the levels of antioxidants in cardiac tissues. AF treatment inhibited the activation of nuclear factor-κB (NF-κB) and decreased the immuno-expression of NF-κB in cardiac tissue. Furthermore, AF caused a marked lowering in the level of inflammatory cytokines (nitric oxide, tumor necrosis factor-α, and interleukin-6) in the cardiac tissue. Collectively, this study demonstrates the cardioprotective activity of AF against DOX-induced cardiac damage which may be due to its antioxidant and anti-inflammatory activities.

1. Introduction

Doxorubicin (DOX) is a highly active chemotherapeutic agent that is still used for the treatment of various types of malignancies despite its deleterious dose-dependent cardiotoxic effects [1]. The exact mechanism of DOX-induced cardiotoxicity is not fully elucidated although many putative pathways have been demonstrated. Studies have demonstrated the fundamental role of reactive oxygen species (ROS) in mediating DOX-induced cardiotoxicity. Administration of DOX causes an overproduction of ROS which results in lipid peroxidation and

Abbreviations: AF, aspernolide F; $CHCl_3$, Chloroform; CK-MB, creatine kinase; MB, isoenzyme; cTnI, troponin I; cTnT, troponin T; DOX, doxorubicin; ESIMS, electron spray ionization mass spectrum; ECG, Electrocardiogram; EtOAc, Ethyl acetate; 4-HNE, 4-hydroxynonenal; GSH, reduced glutathione; H&E, hematoxylin and eosin; H_2SO_4, Sulphuric acid; IL-6, interleukin-6; IL-1β, interleukin-1 beta; iNOS, nitric oxide synthase; LDH, lactate dehydrogenase; MDA, malondialdehyde; MeOH, methanol; NOx, Nitrogen oxides; RAW-264.7, Rat leukemia monocyte macrophage cell line; RP-18 CC, Reversed phase column chromatography; ROS, reactive oxygen species; NF-κB, nuclear factor-kappa B; SOD, superoxide dismutase; TLC, Thin layer chromatography; TNF-α, tumor necrosis factor-alpha; SiO_2, silica gel; VLC, Vacuum liquid chromatography

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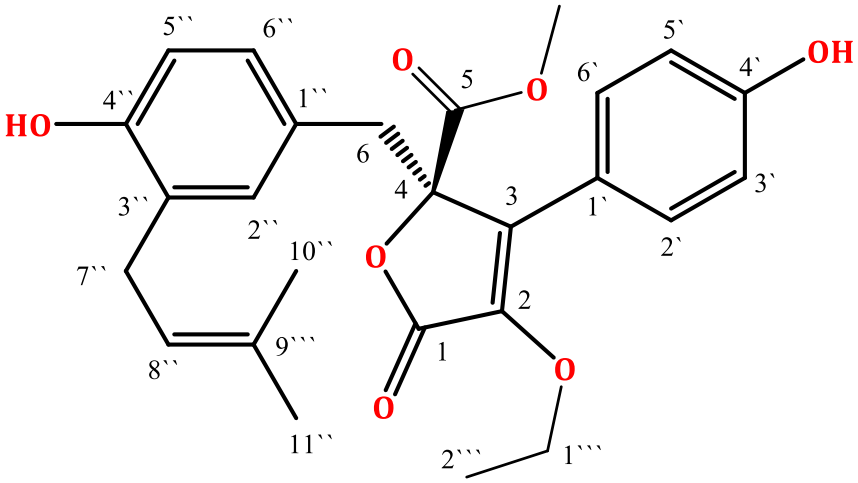

Fig. 1. Chemical structure of aspernolide F (AF).

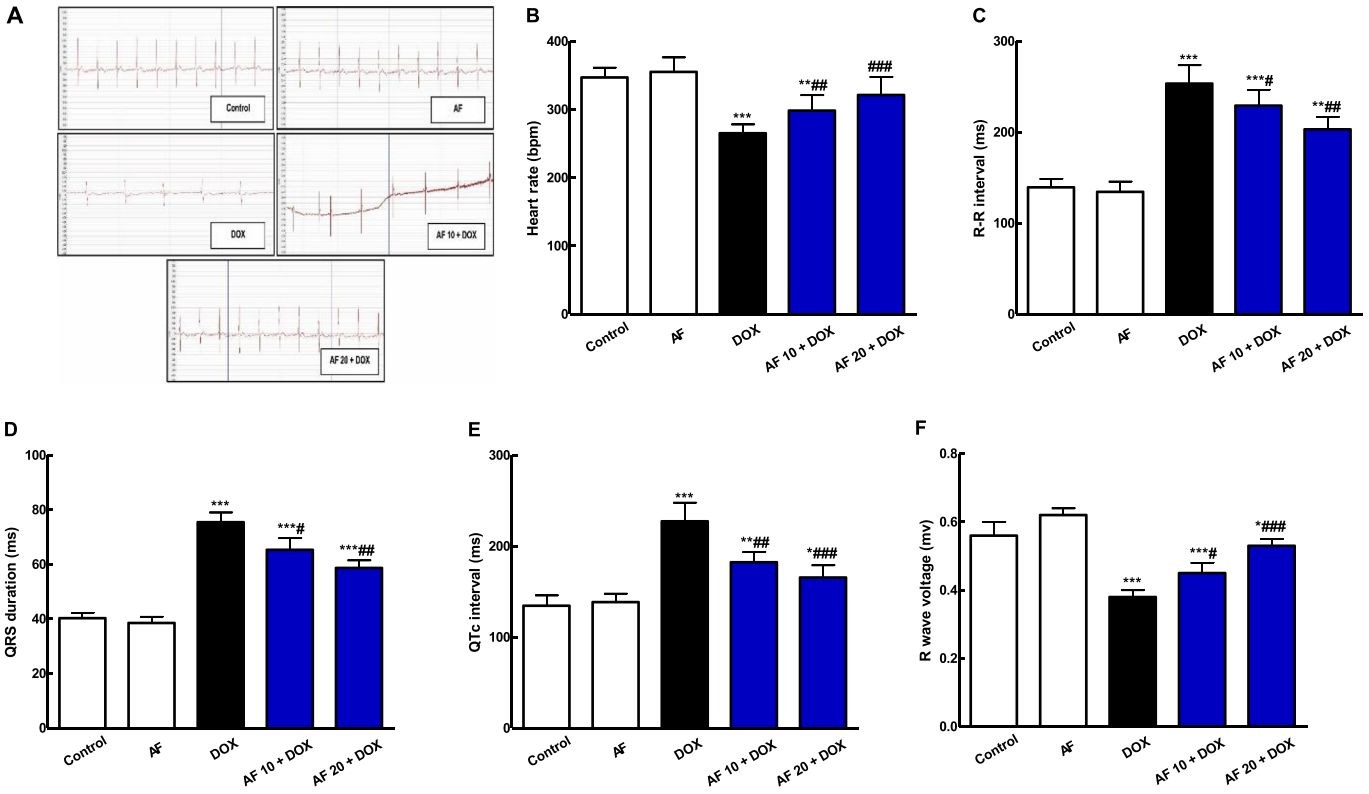

Fig. 2. Effect of aspernolide F (AF) on doxorubicin (DOX)-induced ECG abnormal changes.
A. ECGs recordings; B–F. ECG variables. AF + DOX treated groups were administered AF by oral gavage at two different doses (10 and 20 mg/kg) once daily for 10 days prior to DOX administration. Data are means ± SE (n = 8).
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the DOX group.

subsequent oxidative injury to myocytes [2,3]. DOX-induced oxidative stress has been closely linked to the activation of the transcription factor, nuclear factor-kappa B (NF-κB), which controls the expression of inflammatory cytokines within the myocytes [4,5]. Recently, apoptosis has been shown to be greatly involved in DOX cardiotoxicity [1]. As the clinical use of DOX continues, searching for new therapeutics that could interrupt DOX-induced pathogenic events and confer protection against its cardiotoxicity is continuous.

Endophytic fungi live asymptomatically in most living plants tissues [6]. Recently, attention has been focused on them as one of the most important and underexplored resources for significant bio-metabolites with wide range of pharmaceutical, agricultural, and/or industrial potentials [7,8]. *Aspergillus* is a large diverse genus, including ≈185 filamentous fungal species [9]. It is known to be a wealthy source for γ-butyrolactones, which possess various bioactivities: anti-malarial, cytotoxic, antiviral, antileishmanial, anti-diabetic, anti-microbial, anti-oxidant, anti-cholinesterase, and cyclin-dependent kinases and lipoxygenase inhibitory [6,10,11]. However, no data are available on the cardioprotective activity of γ-butyrolactones. In our continued search to explore bioactivities of the metabolites from fungal source, aspernolide F (AF) was separated from the endophytic fungus *A. terreus* isolated from *Carthamus lanatus* roots and its protective effect against DOX-induced cardiotoxicity in rats was assessed.

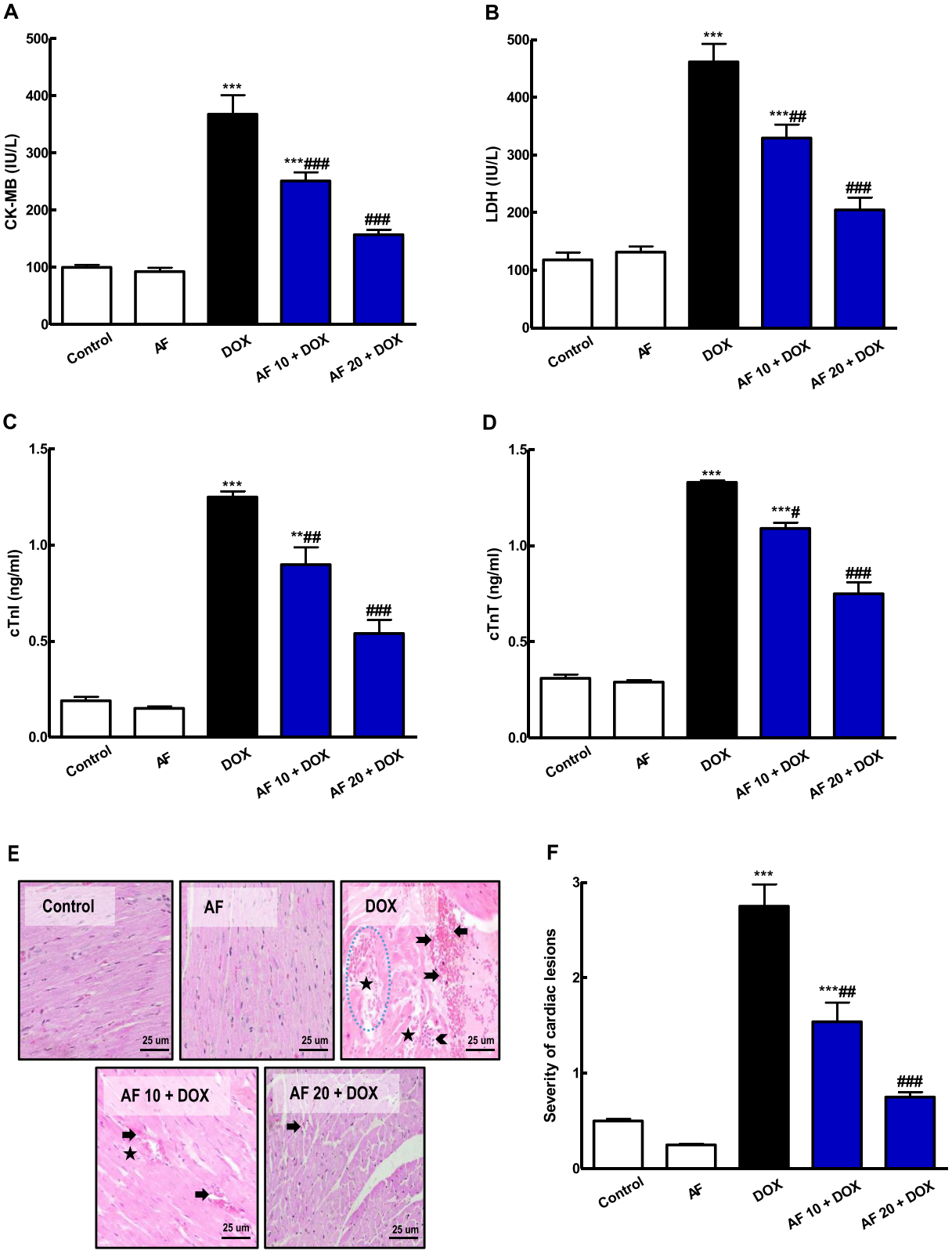

Fig. 3. Effect of aspernolide F (AF) on doxorubicin (DOX)-induced cardiotoxicity.
Serum markers of cardiotoxicity A. Creatine kinase-MB (CK-MB); B. Lactate dehydrogenase (LDH); C. Cardiac troponin I (cTnI); D. Cardiac troponin T (cTnT). E. Histopathology of the heart. Control and AF groups showed normal architecture of the cardiac tissue. Dox group showed marked cardiac lesions as there was coagulative necrosis of cardiac muscles, with loss of striation bands & nuclei (stars), congested blood vessels (thick arrows), interstitial hemorrhage (notched arrows) and inflammatory changes (chevron). AF + DOX treated groups exhibited remarkable improvement of DOX-induced cardiac lesions, H&E stain ×400, scale bar 25 μm. F. The severity of cardiac lesions among different groups. AF + DOX treated groups were administered AF by oral gavage at two different doses (10 and 20 mg/kg) once daily for 10 days prior to DOX administration. Data are means ± SE (n = 8).
$^{**}P < 0.01$, $^{***}P < 0.001$ vs. the control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the DOX group.

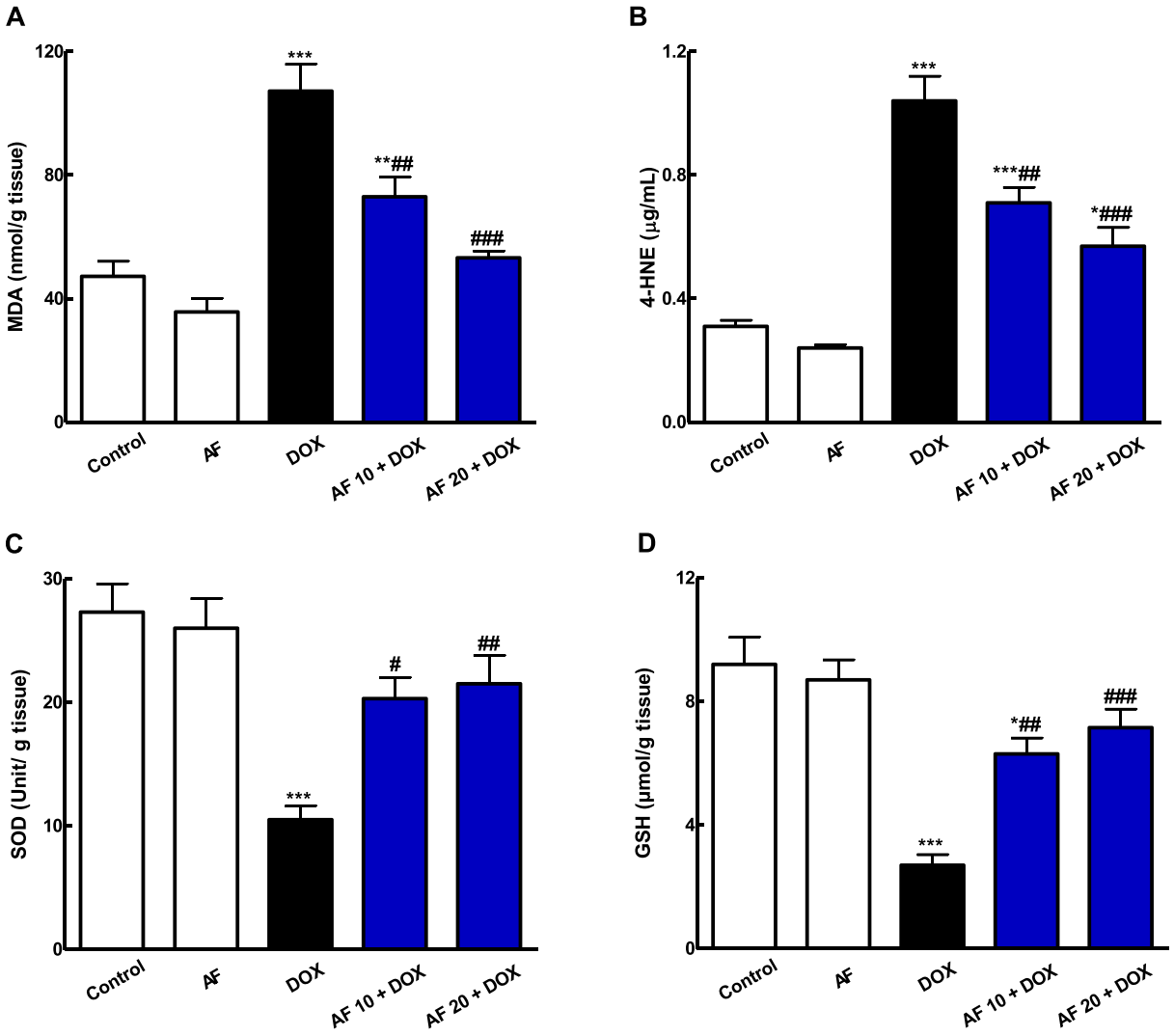

Fig. 4. Effect of aspernolide F (AF) on doxorubicin (DOX)-induced alterations in oxidative stress and antioxidants in cardiac tissue.
A. Malondialdehyde (MDA); B. 4-Hydroxynonenal (4-HNE); C. Superoxide dismutase (SOD); D. Reduced glutathione (GSH). AF + DOX treated groups were administered AF by oral gavage at two different doses (10 and 20 mg/kg) once daily for 10 days prior to DOX administration. Data are means ± SE (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the DOX group.

2. Materials and methods

2.1. General

Positive-ion electron spray ionization mass spectrum (ESIMS) was performed on a Thermo-finnigan LCQ DECA mass spectrometer (Thermo-Finnigan, Bremen, Germany). A 400 MHz Bruker Avance DRX spectrometer was used in measuring NMR spectra (Bruker BioSpin, Massachusetts, USA). Compound separation was performed on SiO_2 60, Sephadex LH-20, and RP-18 (Merck, Darmstadt, Germany). TLC was done on pre-coated SiO_2 60 F_{254} TLC plates (0.2 mm). For detecting compound, UV absorption (λ_{max} 255 and 366 nm) and anisaldehyde:H_2SO_4 reagent were utilized.

2.2. Fungal material

The previously isolated and identified strain of *Aspergillus terreus* (AST No. Feb 2013) was cultivated in 50 Erlenmeyer flasks (1 L each), containing rice solid cultures (100 mL of distilled water was added to 100 g commercially available rice and kept overnight prior to autoclaving) [7,9]. The cultures were then incubated at room temperature for 30 days under septic conditions.

2.3. Metabolites isolation

The culture was extracted using EtOAc and concentrated under vacuum. The obtained extract was suspended in distilled water (200 mL) and fractionated between *n*-hexane and MeOH. The MeOH extract (56.4 g) was separated on normal silica gel (SiO_2) VLC, utilizing *n*-hexane, EtOAc, and MeOH, which were independently concentrated to get FH (4.9 g), FE (37.4 g), and FM (11.8 g), respectively. Fraction FE (37.4 g) was subjected to sephadex LH-20 column chromatography (CC) using $CHCl_3$:MeOH (70:30) to obtain nine sub-fractions: FE-1 to FE-9. Sub-fraction FE-5 (6.2 g) was chromatographed over SiO_2 column (250 g × 100 × 2 cm) using $CHCl_3$:MeOH (98,2 to 80:20) as an eluent to give impure aspernolide F. Its purification was achieved on RP-18 column (0.04–0.063 mm, 100 g, 50 × 2 cm), using H_2O:MeOH gradient (67.5 mg, yellow gum).

2.4. Spectral data of aspernolide F

Yellow powder; $[\alpha]_D$ + 41.3 (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 3.42 (2H, m, H-6), 7.51 (2H, d, *J* = 8.4 Hz, H-2′,6′), 6.89 (2H, d, *J* = 8.4 Hz, H-3′,5′), 6.38 (1H, d, *J* = 2.0 Hz, H-2″), 6.53 (1H, d, *J* = 8.0 Hz, H-5″), 6.49 (1H, dd, *J* = 8.0, 2.0 Hz, H-6″), 3.01 (2H, d,

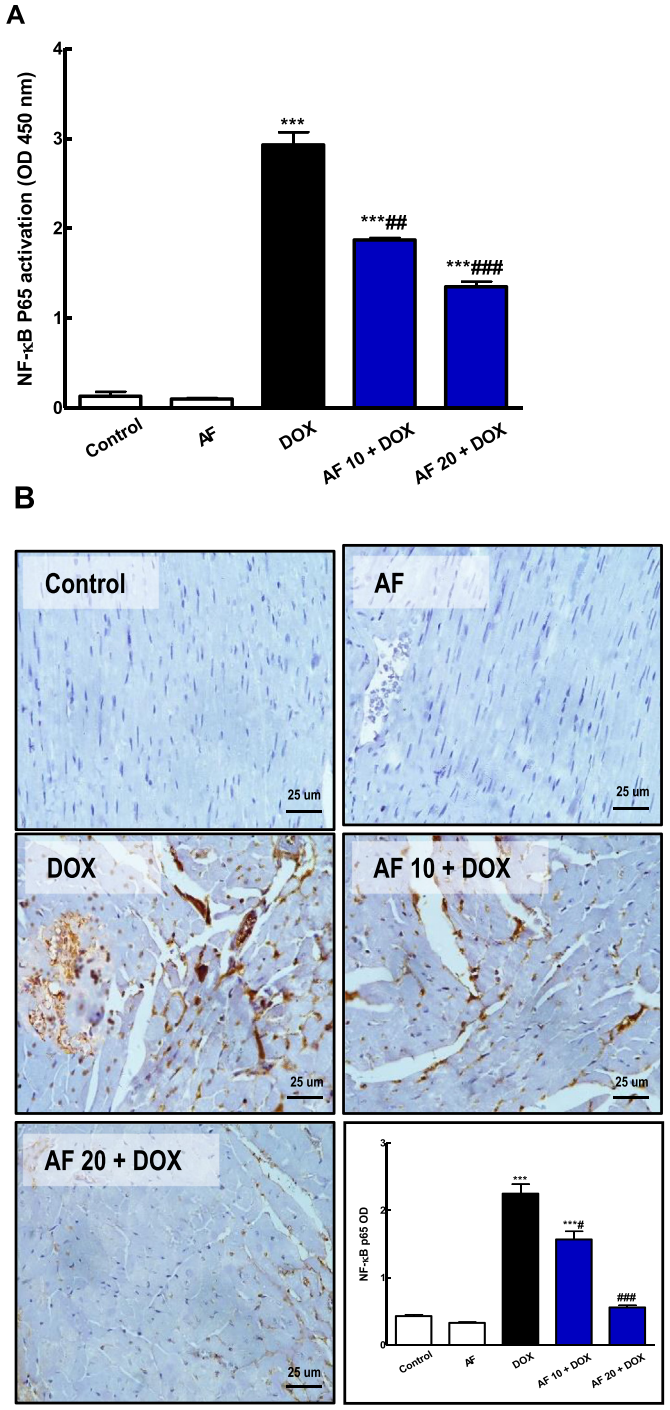

Fig. 5. Effect of aspernolide F (AF) on doxorubicin (DOX)-induced nuclear factor-κB (NF-κB) activation.

A. Level of NF-κBp65 in cardiac tissue. B. Representative NF-κB immunostaining of heart muscle sections (× 400, scale bar 25 μm) where Control and AF groups showed normal heart without any positive immunostaining; DOX group exhibited marked nuclear brown immune-staining; AF + DOX treated animals showed attenuation of the nuclear brownish immunostaining with more prominent effect in the group receiving the higher dose of AF; Semiquantitative analysis of NF-κB IHC staining results expressed as optical densities (OD) across 10 different fields for each TmA section. AF + DOX treated groups were administered AF by oral gavage at two different doses (10 and 20 mg/kg) once daily for 10 days prior to DOX administration. Data are means ± SE (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the DOX group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

J = 7.6 Hz, H-7″), 5.02 (1H, d, J = 7.6 Hz, H-8″), 1.63 (3H, s, H-10″), 1.54 (3H, s, H-11″), 3.75 (3H, s, 5-OCH_3), 4.05 (2H, q, J = 6.5 Hz, H-1‴), 1.18 (3H, t, J = 6.5 Hz), 9.94 (1H, s, 4′-OH), 9.14 (1H, s, 4″OH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 168.4 (C-1), 138.5 (C-2), 128.0 (C-3), 85.2 (C-4), 170.8 (C-5), 38.6 (C-6), 121.5 (C-1′), 129.2 (C-2′,6′), 158.5 (C-4′), 116.2 (C-3′,5′), 123.6 (C-1″), 131.8 (C-2″), 127.0 (C-3″), 154.2 (C-4″), 114.6 (C-5″), 128.9 (C-6″), 28.0 (C-7″), 122.8 (C-8″), 131.4 (C-9″), 25.9 (C-10″), 17.9 (C-11″), 53.9 (5-OCH_3), 60.2 (C-1‴), 14.5 (C-2‴); ESIMS m/z: 453 $[M + H]^+$ [7].

2.5. Animals

Adult male Wistar rats weighing 140–160 g were obtained from animal facility of College of Pharmacy, Taibah University. Rats were kept in standard conditions of temperature, humidity, a 12-h light/dark cycle, and nutrient supplement. The experimental protocol was approved by the Research Ethical Committee of Taibah University which adopts the principles and guidelines for Care and Use of Laboratory Animals of NIH.

2.6. Chemicals

DOX as ampoules (Adriblastina, Pharmacia, Italy) was generously supplied by King Fahd Hospital, Al-Madinah Al-Munawarah, Saudi Arabia. Other chemicals are of high analytical grade.

2.7. Experimental design

Rats were randomly divided into five experimental groups (each of 8 rats). Animals were treated as follows: Control group, rats were orally administered the vehicle once daily for 10 days; AF group where rats were administered AF (20 mg/kg) by oral gavage once daily for 10 days; DOX group where rats were administered the vehicle orally once daily for 10 days and then DOX (20 mg/kg, i.p) on day 10 [4]; two AF + DOX groups that received AF by oral gavage at two different doses (10 and 20 mg/kg) once daily for 10 days before DOX administration.

Forty-eight hours after DOX injection, rats were weighed, anesthetized using ketamine (75 mg/kg, i.p), and subjected to electrocardiography (ECG) recording. Afterwards, blood samples were obtained and centrifuged to collect serum for biochemical analysis. Rats were then humanely sacrificed. Hearts were gathered and washed in ice cold saline. Small piece of each heart was cut, immersed in buffered formalin for 24 h–36 h and embedded in paraffin for histopathological analysis. Another piece of each heart was weighed and homogenized in normal saline then centrifuged to get the supernatants.

2.8. ECG measurements

In lead II position, needle electrodes were inserted under the skin of the animals. ECG was recorded and analyzed using iWorx data recorder and the Labscribe2 software (Model 214, iWorx Systems, Inc., Dover, NH, USA).

2.9. Biochemical measurements

2.9.1. Estimation of serum creatine kinase isoenzyme-MB (CK-MB), lactate dehydrogenase (LDH), troponin I (cTnI), and troponin T (cTnT)

CK-MB level was detected using kinetic photometric method (Eli Tech, Sees, France). The samples were measured at 340 nm using UV–visible spectrophotometer (Model 1200, UNICO Instruments Co., Dayton, NJ, USA). The increase in the absorbance at this wavelength is directly related to the activity of CK-MB and the results were expressed as U/L. LDH activity was measured using kinetic method (Human, Wiesbaden, Germany). Briefly, the reaction mixture (sodium pyruvate, TRIS buffer and NADH) was added to the sample. Absorbance was read

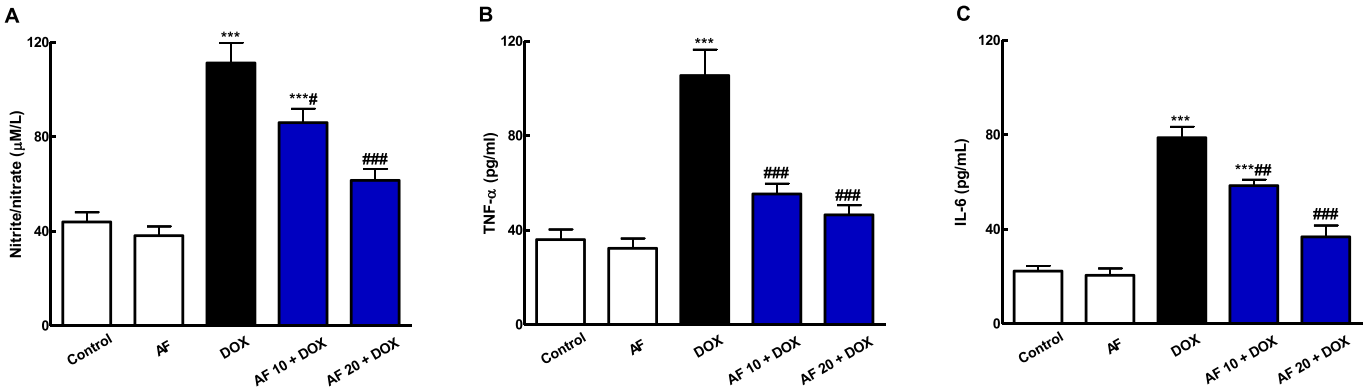

Fig. 6. Effect of aspernolide F (AF) on doxorubicin (DOX)-induced cytokine release in cardiac tissue.
AF + DOX treated groups were administered AF by oral gavage at two different doses (10 and 20 mg/kg) once daily for 10 days prior to DOX administration. Data are means ± SE (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. the DOX group.

at 340 nm and the mean absorbance change per minute was determined. LDH activity was calculated and expressed in U/L. cTnI and cTnT were measured in serum using ELISA kit (Kamiya Biomedical Co, USA).

2.9.2. Estimation of malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), reduced glutathione (GSH), and superoxide dismutase (SOD)

In the supernatants of cardiac homogenates, MDA, GSH and SOD were measured according to the instructions of the kits (Spectrum Co., Egypt). Briefly, MDA is quantified via the interaction between samples and thiobarbituric acid at 95 °C for 30 min to form a pink thiobarbituric acid reactive product which is measured spectrophotometrically at 534 nm. MDA content was expressed as nmol/g tissue. Measurement of SOD activity is based on the ability of SOD to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. The increase in absorbance was measured at 560 nm for 5 min. SOD activity is expressed as Unit/g tissue. GSH is mixed with 5,5-dithiobis-2-nitrobenzoic acid to produce yellow chomogen that its absorbance can be measured at 412 nm. GSH content was expressed as μmol/g tissue. 4-HNE was determined in the supernatants of cardiac homogenates using ELISA kit (MyBioSource Inc., USA).

2.9.3. Estimation of the levels of nitric oxide (NOx), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6)

NOx was determined in the supernatants of the cardiac homogenates based on the procedures of the kit (R&D Systems, USA). In brief, nitrate is initially converted to nitrite by nitrate reductase. The resultant nitrite is detected as an azo dye product of the Griess reaction which involves two-step diazotization reaction to produce the diazonium ion. This ion is coupled to *N*-(1-naphthyl) ethylenediamine to form the chromophoric azo-derivative that can be measured at 540 nm.

These inflammatory cytokines (TNF-α and IL-6) were determined in the supernatants of the cardiac homogenates according to the protocol of the ELISA kits (R&D Systems, USA). ELISA immune assay is based on the interaction between the target cytokine and a monoclonal antibody specific for that cytokine in a microplate.

2.9.4. Estimation of NF-κB p65 activation

Initially, the nuclear extract of the cardiac tissue was obtained using the extraction kit (Abcam, Cambridge, MA, USA). Briefly, small piece of the heart was weighted, homogenized then mixed with extraction buffer containing dithiothreitol (DTT) and incubated on ice for 15 min then centrifuged for 10 min at 4 °C. The supernatant was removed. The nuclear pellets were mixed with extraction buffer containing DTT and protease inhibitor cocktail then incubated-on ice for 15 min with vortex every 3 min. The suspension was further centrifuged at 4 °C and the supernatant was obtained. The protein content of the nuclear extract was measured. NF-κB p65 activation was measured in the nuclear extracts according to the instruction protocol of the kit (Abcam, Cambridge, MA, USA). Results were represented as OD 450 nm.

2.10. Histopathological assessments

The paraffin blocks of cardiac tissue were sectioned and specimen were stained with hematoxylin and eosin (H&E). Histopathological examination was done using light microscope. The severity of myocardial injury was graded according to a semi-quantitative score as described previously [4].

2.11. Immunohistochemical (IHC) assessment

IHC was performed as previously described [4]. In brief, paraffin blocks of cardiac tissues were used to extract mini tissue cores and then tissue miniarray (TmA) were constructed. IHC staining of NF-κB p65 was performed using rabbit polyclonal anti-NF-κB p65 primary antibody and goat anti-rabbit IgG (H + L) secondary antibody (Thermo Fisher Scientific Inc., Waltham, MA, USA). Steps of IHC were done automatically using Ventana Bench Mark XT system (Ventana Medical Systems, Tucson, AZ).

2.12. Statistical analysis

Data were presented as means ± SE for eight rats in each group. Statistical difference among experimental groups was determined using One-way analysis of variance (ANOVA) followed by Tukey's Kramer Multiple Comparison Test. The value of $P < 0.05$ was considered as significant.

3. Results

3.1. Identification of AF

The culture of *A. terreus* was extracted with EtOAc. The extract was subjected repeatedly to SiO_2, sephadex, and RP-18 CC to afford AF a yellow gum that was identified by comparison of its physical and spectral data with the previously reported data [7] (Fig. 1).

3.2. Effect of AF on DOX-induced ECG changes

DOX injection resulted in a significant bradycardia compared to normal animals (Fig. 2A). Additionally, significant increase in QTc interval, ST segment elevation and R wave amplitude was observed in ECG of DOX group (Fig. 2B–E). But ECG abnormal changes were significantly attenuated in AF treated animals.

3.3. Effect of AF on serum markers of DOX-induced cardiotoxicity

As shown in Fig. 3A–D, DOX administration provoked elevation in serum levels of CK-MB, LDH, cTnI, and cTnT compared to the control group. AF pretreatment resulted in a significant amelioration of these serum markers of cardiac damage compared to DOX group.

3.4. Effect of AF on DOX-induced pathological damage of the cardiac tissue

The histopathological examination confirmed the presence of cardiac lesions in DOX treated animals compared to normal architecture of the heart. The lesions were in the form of necrotic and inflammatory changes in the cardiac tissue in addition to loss of myocardial striation. Remarkably, AF treatment preserved the integrity of the cardiac tissue. AF notably prohibited the development of DOX-induced cardiac lesions (Fig. 3E and F).

3.5. Effect of AF on DOX-induced lipid peroxidative changes in cardiac tissue

As shown in Fig. 4A and B, DOX injection caused an increase in the lipid peroxidative products, MDA and 4-HNE in the cardiac tissue. Also, DOX significantly decreased the antioxidants, GSH and SOD in the heart (Fig. 4C and D). However, AF administration repressed the increase of these lipid peroxidative markers and restored the normal level of the antioxidants in the cardiac tissue compared to DOX group.

3.6. Effect of AF on DOX-induced activation of NF-κB

DOX injection resulted in activation of NF-κB signaling pathway. NF-κB level as well as NF-κB p65 immuno-positive cells were significantly elevated in DOX group in comparison to control animals (Fig. 5A and B). On the other hand, AF pretreatment significantly hindered the activation of NF-κB as it decreased the level of NF-κB p65 and ameliorated its immuno-positive expression (Fig. 5).

3.7. Effect of AF on DOX-induced cytokines release

The levels of the inflammatory cytokines (NOx, TNF-α, and IL-6) were greatly increased in DOX group compared to control group (Fig. 6A–C). However, AF effectively lowered the levels of NOx, TNF-α, and IL-6 compared to DOX group.

4. Discussion

DOX-induced cardiotoxicity is still a significant obstacle to its clinical use in oncology that encourage a continuous search for new therapeutics that could hinder the injurious effects of DOX. This study evokes the cardioprotective activity of the BL derivative, AF, against DOX-induced cardiotoxic effects which may be related to its anti-inflammatory and antiapoptotic activities.

Results of the present study revealed that a single dose of DOX results in deleterious effects on cardiac tissue. The functional abnormalities were seen in DOX-induced ECG changes as there was a decrease in heart rate, prolongation of QRS duration, R-R interval, QTc interval, and a significant decrease in the R wave voltage. These findings are like those reported previously [4,12]. AF administration ameliorated DOX-induced ECG changes and to some extent restored normal ECG rhythm in a dose dependent manner. Biochemical measurements of the serum markers of cardiac lesions (CK-MB, LDH, cTnI, and cTnT) confirmed the occurrence of sever cardiotoxic effects in DOX group. These results are in accordance with the previous studies [13]. It has been demonstrated that DOX induces reactive oxygen species (ROS) generation which causes membrane peroxidation and death of cardiomyocytes. Consequently, cytoplasmic markers as CK-MB and LDH are leaked into the blood [14]. AF pretreatment resulted in a dose-dependent decrease in the release of these markers into the serum suggesting that AF protected the integrity of cardiomyocytes. The results of the histopathological examination were in the same line with ECG and biochemical analysis. Degenerative changes of the heart were seen in DOX group which were remarkably improved in AF treated animals. Collectively, these results suggested that AF had a remarkable cardioprotective effect against DOX-induced cardiac injury.

Multiple pathogenic events are implicated in DOX-induced cardiotoxicity. As described previously DOX administration resulted in increased levels of ROS which induced oxidative damage to cellular components and death of cardiomyocytes. Additionally, DOX has a high affinity for the phospholipid component of mitochondrial membrane in cardiomyocyte, leading to DOX accumulation in cardiac tissue which in turn is characterized by high susceptibility to oxidative injury as compared to other organs due to low levels of free-radical detoxifying enzymes/molecules like SOD, GSH, and catalase [2,15,16]. Our results were in the same direction of the previous studies as it confirmed that DOX increased the level of peroxidative markers (MDA and 4-HNE) in cardiac tissues and depressed the antioxidants capacity of the heart. Conversely, AF pretreatment enhanced the antioxidants and suppressed the peroxidative markers in the heart tissue. These results may partially explain the molecular mechanism of cardioprotective effects of AF.

In addition to its direct toxic effects, ROS can induce the activation of the transcription factor, NF-κB leading to its translocation into the nucleus where it binds to the promoter elements and activate the expression and production of inflammatory cytokines such as TNF-α, ILs, and NOx [4,17,18]. The results of the present study revealed that DOX injection caused activation of NF-κB with subsequent increase in the expression and production of inflammatory cytokines such as NOx, TNF-α and IL-6. These results were in harmony with the previous investigations that reported the ability of DOX to induce potent inflammatory response through activation NF-κB [1,18].

Collectively, this study provides a piece of evidence of the cardioprotective activity of AF against acute DOX-induced cardiac damage. This effect may be mediated through the antioxidant activity of AF which suppressed oxidative stress. It is noteworthy that the antioxidant potential of the γ-butyrolactones derivatives has been previously demonstrated [11]. Iqbal et al. reported that the presence of the butyrolactone ring played an important role in the inhibition of TNF-α production [19]. Also, Zhang et al. stated that AF analogue, butyrolactone I down-regulated the production of nitric oxide (NO), interleukin-1 beta (IL-1β), and inducible nitric oxide synthase (iNOS) and inhibited the phosphorylation of nuclear factor-κB (NF-κB) and inhibitor of NF-κB (I κB) in BV-2 cells [20]. It also exhibited the inhibitory effect on NO which was close to indomethacin [21]. Moreover, asperteretal A significantly decreased the NO production in RAW264.7 cells [22].

5. Conclusion

Our results suggested that AF may act as a potential cardioprotective candidate against DOX-induced cardiotoxicity due to its antioxidant and anti-inflammatory activities. Thus, these data provide a new insight into the secondary metabolites derived from endophytic fungi. However, further researches are needed for a deeper elucidation of the molecular pathways of AF.

Conflict of interest

The authors declare to have no conflict of interest.

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