

Original Article



Possible Cardio-protective Effects of TND1128 and Omega-3 Fatty Acids Against Daunorubicin-Induced Cardiotoxicity in Male Wistar Rats: A Comparative In Vivo Study

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Abstract

Background: Daunorubicin, an anthracycline antibiotic widely used in chemotherapy, is limited due to its dose-dependent cardiotoxicity. Omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) provide cardioprotective benefits through reducing oxidative damage, suppressing inflammatory markers and enhancing antioxidant defenses. TND1128, a novel 5-deazaflavin derivative with mitochondrial-targeted redox activity, has been studied in hepatic and neuronal oxidative stress models, but its cardioprotective potential remains unexplored. This study aimed to evaluate the efficacy of Omega-3 and TND1128 in preventing daunorubicin-induced cardiotoxicity.

Methods: Thirty-six male Wistar rats were randomized into six groups: Group I received corn oil; Group II received daunorubicin (12 mg/kg i.p., last 3 days; cumulative dose 36 mg/kg); Group III received omega-3 (600 mg/kg/day, orally, 14 days); Group IV received omega-3 (600 mg/kg/day, by oral gavage, 14 days)+daunorubicin (12 mg/kg i.p., last 3 days); Group V received TND1128 (10 mg/kg/day, by oral gavage, 14 days), and group VI received TND1128 (10 mg/kg/day, by oral gavage, 14 days)+daunorubicin (12 mg/kg i.p., last 3 days). Cardiac injury indicators, oxidative stress markers, inflammation mediators, apoptotic signaling components (caspase-3), and histopathological analysis were assessed.

Results: Both interventions significantly attenuated daunorubicin-induced biochemical, molecular, and histological alterations ($P < 0.01$). TND1128 showed greater efficacy in reducing apoptosis and inflammation compared to omega-3 fatty acids, highlighting its potential as an adjunct therapy in anthracycline-based regimens.

Conclusion: Omega-3 fatty acids and TND1128 significantly protected against daunorubicin-induced cardiotoxicity by improving oxidative stress, inflammation, apoptosis, and myocardial integrity. TND1128 provided superior protection, supporting its potential as an adjunct therapeutic strategy in anthracycline chemotherapies.

Introduction

Anthracyclines, such as daunorubicin, remain key chemotherapeutic agents for treatment of leukemias, lymphomas, and solid tumors due to their potent cytotoxicity mediated through DNA intercalation, topoisomerase II inhibition, and reactive oxygen species (ROS) generation.¹ Despite their potent anticancer efficacy, daunorubicin's therapeutic efficacy is significantly limited by dose-dependent cardiotoxicity, which may occur acutely (e.g., arrhythmias or myocarditis) or chronically, leading to irreversible congestive heart failure years after therapy. This cardiotoxic effect is attributed to cumulative myocardial injury driven by oxidative stress, mitochondrial dysfunction, and inflammation.²

Daunorubicin-induced cardiotoxicity has several mechanisms, including oxidative stress, mitochondrial

dysfunction, DNA damage, and activation of apoptotic and inflammatory pathways.³ A critical pathogenic process involves the redox cycling of the quinone moiety of anthracycline in the presence of iron, which produces superoxide anions and hydroxyl radicals via the Fenton reaction. These ROS induce progressive structural and functional degradation of cardiac tissue by damaging cellular membranes, proteins, and nucleic acids.⁴

The only FDA-approved cardioprotective agent for anthracycline-induced cardiotoxicity is dexrazoxane.⁵ As a result, given the serious limits of current cardioprotective drugs, there is an increasing need for safer and more effective cardioprotective therapies to protect against anthracycline-induced heart injury.

Omega-3 polyunsaturated fatty acids (PUFAs), found in marine oils and seafood, play a fundamental role in

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neurological function and progression.⁶ They have shown beneficial effects in several chronic degenerative diseases such as cardiovascular disease, rheumatoid arthritis, other autoimmune disorders, cancer, and diabetes.⁷ Omega-3 PUFAs (as docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]) dietary supplements have numerous therapeutic benefits when administered prior to or during the cancer treatment. These include reversing the drug resistance of the tumor cell, reducing hematological, cardiac and gastrointestinal side effects of chemotherapeutic agents, preventing alopecia, and reducing cancer cachexia.^{7,8} In experimental models of doxorubicin-induced cardiomyopathy, omega-3 supplementation significantly reduced lipid peroxidation, inhibited NF- κ B signaling, and protecting against both structural and functional damage of the myocardium associated with doxorubicin cardiotoxicity.⁹

TND1128, a 5-deazaflavin derivative (10-ethyl-3-methylpyrimido [4,5-*b*]quinoline-2,4(3*H*,10*H*)-dione), is a flavin analogues synthesized to mimic nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) redox functions.¹⁰ TND1128 is an activator of mitochondrial energy synthesis and has recently attracted attention for its potent redox-modulatory and mitochondrial-stabilizing properties, especially in neurons. It exhibits superior chemical stability and enhanced electron transfer capacity, enabling efficient scavenging of ROS. Furthermore, TND1128 preserves mitochondrial membrane potential and calcium handling, thereby that protecting neurons from deterioration.¹¹ However, its potential cardioprotective efficacy remains largely unexplored, necessitating further targeted investigations in cardiac tissues.

The current study is designed to investigate the potential protective effects of TND1128 (a 5-deazaflavin derivative) in comparison with omega-3 PUFAs in rats against daunorubicin-induced cardiotoxicity

Methods

Reagents and Kits

Daunorubicin hydrochloride (Dauneon[®], 20 mg/vial, lyophilized powder for intravenous use) was obtained from NEON Laboratories Ltd., Mumbai, India. Omega-3 fish oil concentrate (Solgar[®], 120 softgels, purified to remove mercury, gluten-, wheat-, and dairy-free) was purchased from Solgar Inc., USA. TND1128 (5-deazaflavin) was sourced from Shandong Longilab Biotechnology Co., Ltd. Corn oil was purchased from Macklin Biochemical Co., Ltd., Shanghai, China, and used as a vehicle for oral administration, in accordance with prior reports demonstrating its safety.¹²

ELISA kits: Rat ELISA kits for troponin I, creatine kinase MB (CK-MB), malondialdehyde (MDA), glutathione (GSH), and nuclear factor kappa B (NF- κ B) were purchased from SunLong Biotech Co., Ltd. (Zhejiang, China).

Western blotting reagents: Anti-Caspase-3 and anti- β -actin antibodies were provided from Abcam (UK).

Additional reagents for blotting procedures (RIPA buffer, inhibitors, chemiluminescent substrate) were obtained from Bio-Rad (USA).

RT-PCR kits: Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, USA), cDNA synthesis was performed using RevertAid First Strand cDNA Synthesis Kit, and gene quantification was carried out using the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific).

Preparation of Solutions

TND1128 was freshly prepared each day by dissolving 40 mg of pure TND1128 powder in 16 mL of corn oil and administering orally via gavage at a dose of 10 mg/kg body weight. Daunorubicin vial was diluted in normal saline and injected intraperitoneally.

Animals and Experimental Design

Thirty-six adults male Wistar rats (150–200 g) were obtained from the Animal House of College of Pharmacy, University of Baghdad. The study was conducted from February 2025 to March 2025. The institutional animal care and Use Committee of the College of Pharmacy, University of Baghdad approved the protocol of this study, and the work was conducted under controlled conditions in accordance with conventional laboratory ethics. Experimental rats were kept in stainless steel cages at 25 °C, also natural light/dark cycle, and with controlled relative humidity. Rodents had free access to tap water and chow ad libitum according to laboratory standards, and before the experiment, the animals were adapted for about one week.

The animals were then divided into six groups of six rats, each as follows:

- Group I (control group): received corn oil (4 mL/kg) orally for 14 successive days by oral gavage.
- Group II (daunorubicin /induction group): received corn oil (4 mL/kg) orally for 14 successive days by oral gavage and subsequently injected with daunorubicin (12 mg/kg) for the last 3 days, with cumulative dose of 36 mg/kg by intraperitoneal injection.¹³
- Group III (Omega-3 fish oil treated): received omega-3 fish oil at a dose of 600 mg/kg/day for 14 successive days by oral gavage.
- Group IV (Omega-3 fish oil + daunorubicin): received an oral dose of omega-3 fish oil 600 mg/kg/day by oral gavage for 14 successive days and subsequently injected with daunorubicin (12 mg/kg) for the last 3 days with cumulative dose 36 mg/kg, by intraperitoneal injection.
- Group V (TND1128 treated): received an oral dose of TND1128 (10 mg/kg) by oral gavage for 14 successive days.
- Group VI (TND1128 + daunorubicin): received an oral dose of TND1128 (10 mg/kg) by oral gavage for 14 successive days and subsequently injected with daunorubicin (12 mg/kg) for the last 3 days by intraperitoneal injection.

Biochemical Assessment of Blood Samples

At the end of the experiment (day 15), animals' blood was drawn from the jugular vein (near the throat or neck) under diethyl ether anesthesia. Whole blood was collected in a serum separator tube and allowed to clot for 30 minutes at room temperature. The samples were then centrifuged at 3000 rpm for 15 minutes to obtain serum, which was transferred to tubes and stored at -20 °C until use¹⁴ for the estimation of troponin I, CK-MB, and NF-κB by the ELISA technique. The process began with the addition of standards, samples, and the blanks to antibody-coated wells. After incubation, unbound Streptavidin-HRP was washed away during a washing step. A substrate solution was then added, and color developed in proportion to the specific enzyme measured in the sample. The reaction was terminated by the addition of an acidic stop solution, and absorbance was measured at 450 nm using a microplate reader.¹⁵

Cardiac Tissue Analysis

After the animals were euthanized, the heart was rapidly excised, cleaned, and washed with extremely cold PBS (pH 7.4, 4 °C). A 10% cardiac tissue homogenate was prepared by adding 0.1 g of the minced tissue and 0.9 mL of phosphate-buffered saline (PBS) (pH 7.4, 4 °C) into a 2 mL microcentrifuge tube, followed by homogenization with a tissue homogenizer at setting 3 for 1 minute at 4 °C. The homogenate was then thawed at 4 °C and centrifuged in a refrigerated centrifuge for 10 minutes at 10000 rpm and 4 °C. The resultant supernatant was rapidly collected, and all samples were stored at -20 °C until analysis.¹⁶

The heart tissue homogenate was utilized for the estimation of MDA and GSH by ELISA technique, inflammation mediators i.e., TNFα (Tumor Necrosis Factor-alpha) and IL-13 by RT-qPCR, and cleaved caspase-3 using Western blotting. Fifty mg of heart tissue were taken and put in the TRIzol reagent, then centrifuged at 12000 × g for 5 min at 4 °C. The resultant aqueous colorless supernatant (containing RNA) was collected. After RNA extraction from all heart tissue samples through several addition and washing steps, RNA concentration was measured using a nano-drop-once apparatus, and all samples with concentration above 2 ng/μL were measured. RNA was then converted to cDNA and stored at 20 °C for subsequent reaction steps.

The RNA amplification reaction was performed using the Rotoe-Gene Q 96 System Software (Version 2.1, Qiagen). The quantity of mRNA was normalized with the expression of beta-actin, and all comparison data were calculated using the 2^{-ΔCt}.

HMOX-1 gene primer sequence for real-time PCR is as follows:

- Forward primer (5'–3') is CTTTCAGAAGGGTCA GGTGTC.
- Reverse primer (5'–3') is TGCTTGTTTCGCTCTA TCTCC.¹²

Western blotting was utilized to determine cleaved

caspase-3 in cardiac tissue homogenate.

The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) method was used to estimate the gene expression levels of TNFα and IL-13, relative to the housekeeping gene GAPDH as a reference gene, in cardiac tissue samples. The assay briefly involved total RNA extraction and purification, cDNA preparation, measurement of gene expression, and data analysis.¹⁷

Histological Examination

The animals' heart tissue was fixed in 10% formaldehyde solution, then paraffin-embedded tissue blocks were cut into 5 μm sections using a microtome, washed in a water bath, and placed in the oven for dewaxing. Sections were then stained with hematoxylin and eosin (H&E) dye and examined under the light microscope by the histopathologist.¹²

Statistical Analysis

All quantitative data were expressed as mean ± standard deviation (SD). Statistical significance was evaluated using one-way analysis of variance (ANOVA) for multiple comparisons, followed by Tukey's test. All graphical plots were generated using GraphPad Prism 10 (San Diego, CA, USA). A p-value of <0.05 was considered statistically significant.

Results

Biochemical Analysis

The daunorubicin group (Group II) showed a significant elevation in serum levels of troponin I, CK-MB, NF-κB, as well as in cardiac tissue levels of MDA, TNF-α, IL-13 ($P < 0.0001$) and cleaved caspase-3 ($P < 0.01$), while showing a significant reduction in cardiac tissue levels of GSH ($P < 0.0001$) compared to the corn oil, the control group (Group I) (Figures 1–8).

Treatment with omega-3 in combination with daunorubicin (Group IV) resulted in a significant reduction in serum levels of troponin I, CK-MB, NF-κB ($P < 0.0001$) and in cardiac tissue levels of MDA, cleaved caspase-3 ($P < 0.0001$), as well as TNF-α and IL-13 ($P < 0.01$), while showing no significant differences in cardiac tissue levels of GSH ($P > 0.05$) compared to the daunorubicin group (Group II) (Figures 1–8).

Treatment with either TND1128 in combination with daunorubicin (Group VI) resulted in a significant reduction in serum levels of troponin I, CK-MB, and NF-κB ($P < 0.0001$), and in cardiac tissue levels of MDA, TNF-α, and cleaved caspase-3 ($P < 0.0001$), and IL-13 ($P < 0.01$), while showing a significant elevation in cardiac tissue levels of GSH ($P < 0.01$) compared to the daunorubicin group (Group II) (Figures 1–8).

Treatment with omega-3 alone (Group III) resulted in no significant differences in serum troponin I, CK-MB, and NF-κB levels, or in cardiac tissue levels of GSH, TNF-α, IL-13 and cleaved caspase-3 ($P > 0.05$), while showing a significant elevation in cardiac tissue levels of

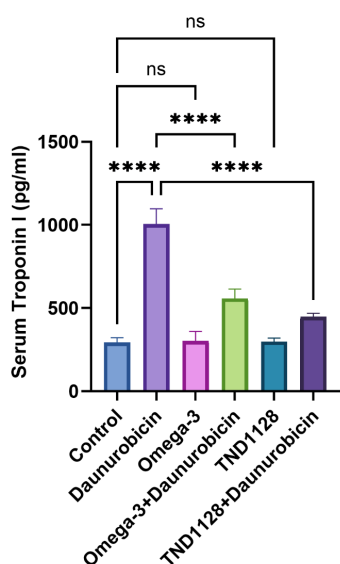


Figure 1. The effect of omega-3 and TND1128 on the serum levels of troponin I in daunorubicin-induced cardiotoxicity. Data are presented as mean $\pm$ SD (n=6). symbol ns=non-significant; * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ (significant)

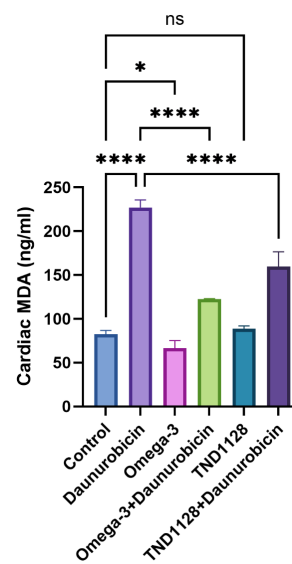


Figure 3. The effect of omega-3 and TND1128 on the cardiac tissue levels of MDA in daunorubicin-induced cardiotoxicity. Data are presented as mean $\pm$ SD (n=6). Symbol: ns: non-significant; * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ (significant)

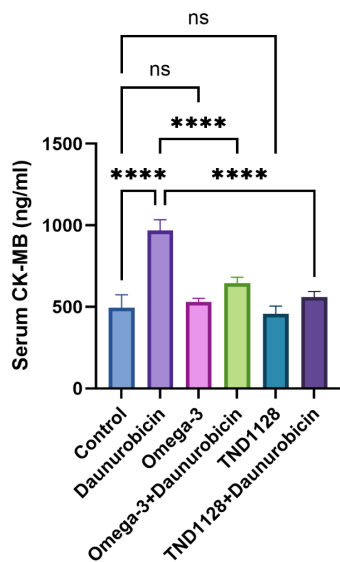


Figure 2. The effect of omega-3 and TND1128 on the serum levels of CK-MB in daunorubicin-induced cardiotoxicity. Data are presented as mean $\pm$ SD (n=6). Symbol: ns=non-significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ (significant)

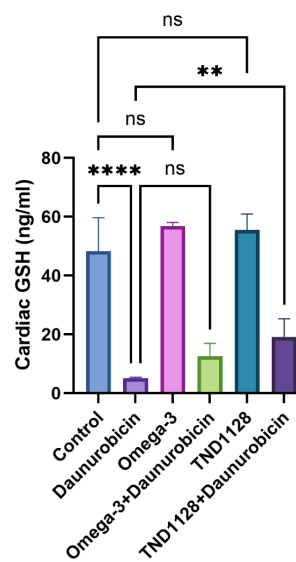


Figure 4. The effect of omega-3 and TND1128 on the cardiac tissue levels of GSH in daunorubicin-induced cardiotoxicity. Data are presented as mean $\pm$ SD (n=6). Symbol: ns: non-significant; * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ (significant)

MDA ($P<0.05$), compared to the corn oil control group (Group I) (Figure 1).

Treatment with TND1128 alone (Group V) resulted no significant differences in serum troponin I, CK-MB, and NF- κ B levels, or in cardiac tissue levels of MDA, GSH, TNF- α , IL-13 ($P>0.05$), while showing a significant reduction in cardiac tissue levels of cleaved caspase-3 ($P<0.05$) compared to the corn oil control group (Group I) (Figures 1–8).

Histopathological Evaluation

Cardiac tissue sections in the corn oil control group (Group I) showed preserved histoarchitecture, with orderly arranged myocardial fibers, centrally located nuclei,

and intact interstitial spaces. No signs of inflammation, necrosis, or edema were detected (Figure 9A).

Cardiac tissue sections from rats treated with daunorubicin (Group II) exhibited pronounced myocardial damage. Extensive necrosis, vacuolar degeneration, and prominent myofiber splitting were evident. Vascular congestion, interstitial edema, and fat droplet accumulation were observed, indicating drug-induced cardiotoxicity (Figure 9B).

Cardiac tissue sections in omega-3 treated group (Group III) showed mild focal myocardial infarctions and moderate atrophy of muscle fibers. There was a slight increase in connective tissue within the perimysial area. Occasional focal hemorrhages were present, likely representing

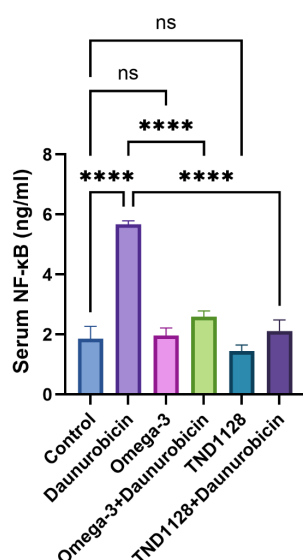


Figure 5. The effect of omega-3 and TND1128 on the serum levels of NF-κB in daunorubicin-induced cardiotoxicity. Data are presented as mean ± SD (n=6). Symbol: ns: non-significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (significant)

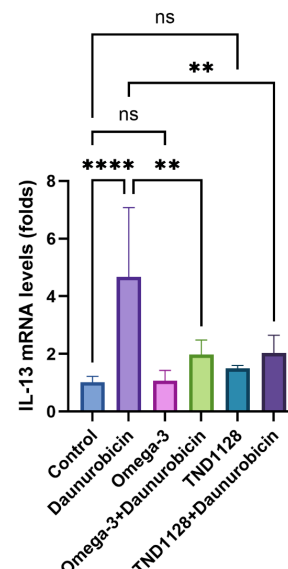


Figure 7. The effect of omega-3 and TND1128 on IL-13 mRNA expression in daunorubicin-induced cardiotoxicity. Data are presented as mean ± SD (n=6). Symbol: ns: non-significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (significant)

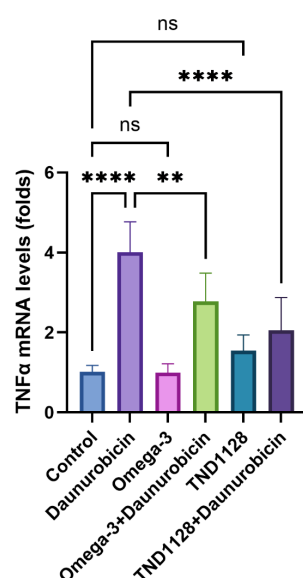


Figure 6. The effect of omega-3 and TND1128 on TNF-α mRNA expression in daunorubicin-induced cardiotoxicity. Data are presented as mean ± SD (n=6). Symbol: ns: non-significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (significant)

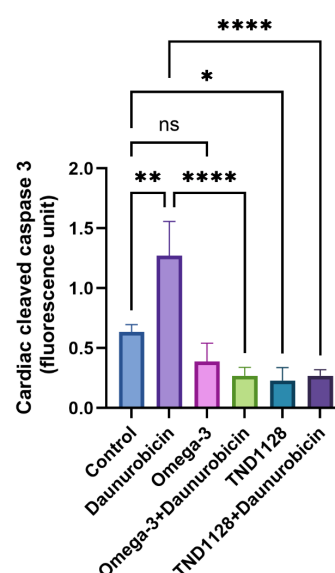


Figure 8. The effect of omega-3 and TND1128 on Cardiac tissue levels of cleaved caspase 3 in daunorubicin-induced cardiotoxicity. Data are presented as mean ± SD (n=6). Symbol: ns: non-significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (significant)

transient microvascular instability (Figure 9C).

Co-administration of omega-3 with daunorubicin (Group IV) demonstrated partial cardioprotection. Histological sections revealed only mild myofiber atrophy and limited connective tissue proliferation. Some slides showed near-normal morphology, suggesting a mitigating effect of omega-3 against daunorubicin-induced damage (Figure 9D).

Cardiac tissue sections from the TND1128 treated group (Group V) appeared largely normal. Minor vascular congestion, mild edema, and rare hemorrhagic spots were observed, suggesting good cardiac tolerance and minimal tissue reactivity to the compound (Figure 9E).

Cardiac tissue sections from co-administration of TND1128 with daunorubicin (Group VI) exhibited largely preserved myocardial structure. However, some areas showed focal hemorrhages and an increase in perimysial connective tissue (Figure 9F).

Discussion

The clinical use of daunorubicin has been limited due to its cardiotoxicity.³ Elevated blood concentrations of cardiac biomarkers are indicative of cardiac injury, which may occur as a consequence of hemodynamic stress, supply-demand imbalance, or direct toxic effects.¹⁷ Troponin is a protein complex that regulates contraction of the striated

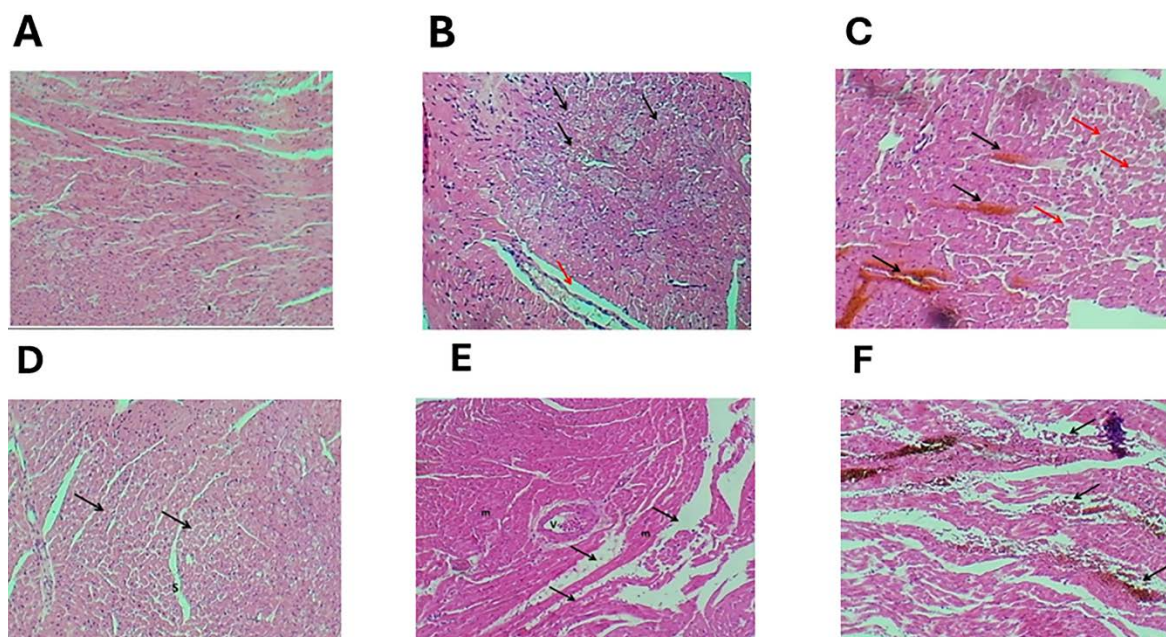


Figure 9. The effect of omega-3 and TND1128 on histological changes of cardiac tissue in daunorubicin-induced cardiotoxicity in rats. Image of H&E-stained cardiac tissue sections (100× magnification) at 24 h after daunorubicin administration are shown: (A) Control group: normal appearance; no histological changes were observed, (B) Daunorubicin group: myocardial infarction (black arrow) and vascular congestion (red arrow), (C) Omega-3 treated group: mild focal infarction (black arrow), (D) Omega-3 + daunorubicin group: mild atrophy (black arrow) with preserved architecture, (E) TND1128 treated group: normal myocardial alignment and minimal congestion (black arrow), (F) TND1128 + daunorubicin group: normal structure with a hemorrhagic focus (black arrow)

muscles. A variety of conditions have been associated with elevated levels of circulating cardiac troponins, such as myocarditis or left ventricular hypertrophy, which are known as asymptomatic heart failure precursors.¹⁷ Elevation of the cardiac troponins is a sign of myocardial tissue damage rather than infarction.¹⁸ Troponin I and T are specific to the heart, while troponin C can also be expressed in skeletal muscle.^{17,19}

Creatine kinase is a dimeric enzyme composed of two subunits, M and/or B, forming three different isoenzymes (CK-BB, CK-MB and CK-MM). The heart-specific isoenzyme is CK-MB, which is considered the gold standard method for acute myocardial infarction (AMI) diagnosis in many laboratories. CK-MB is the second-best marker when troponin assays are not available.²⁰

The present study showed that daunorubicin significantly increases the serum levels of troponin I and CK-MB levels (Figures 1 and 2, respectively) indicating the myocardial damage induced by daunorubicin, as demonstrated by other studies.^{3,21} Moreover, the present study showed that omega-3 in combination with daunorubicin in rats (Group IV) resulted in a significant reduction in serum levels of troponin I and CK-MB, indicating the cardioprotective effects of omega-3 fatty acids, consistent with other studies.^{22,23} Furthermore, the role of cardioprotective effects of TND1128 has not been previously described; however, in this study, TND1128, a 5-deazaflavin derivative, in combination with daunorubicin in rats (Group VI), resulted in a significant reduction in serum levels of troponin I and CK-MB, indicating the cardioprotective effects of TND1128 in male Wistar rats.

The results of the present study revealed a significant increase in MDA levels and a simultaneous depletion of GSH levels in cardiac tissue in the daunorubicin group (Group II), indicating enhanced lipid peroxidation and diminished antioxidant defense. These outcomes are consistent with the established mechanism of daunorubicin cardiotoxicity, where redox cycling of the quinone moiety leads to the generation of ROS and oxidative damage to cellular membranes and organelles.^{3,24}

Treatment with omega-3 fatty acids and TND1128, both alone and in combination with daunorubicin, reinstated GSH levels (except for the omega-3 + daunorubicin group (Group IV) that showed a non-significant increase in GSH levels compared to the daunorubicin group) and significantly reduced MDA concentrations. These results suggest effective antioxidant action. The antioxidative action of omega-3 is likely mediated through incorporation into membrane phospholipids and modulation of redox-sensitive transcription factors,^{6,7,25,26} whereas TND1128, and other 5-deazaflavin analogs have been shown to significantly improve the cell viability against oxidative stress caused by hydrogen peroxide (H_2O_2). TND1128 and its analogs may act through ROS scavenging.²⁷ Mitochondrial respiratory chain dysfunction may consequently increase oxidative stress.²⁸ TND1128 serves as a mitochondrial-stimulating agent, capable rescuing deteriorating neurons in aging or disease conditions.²⁷

NF- κ B sustained activity can perpetuate inflammation and contribute to the pathogenesis of many chronic conditions. Daunorubicin induces the activation of nuclear factor-kappa B (NF- κ B), which enhances the transcription of pro-inflammatory mediators such as

TNF- α , exacerbating the inflammatory cascade and worsening myocardial injury.²⁹

A proinflammatory cytokine, Interleukin (IL)-13, is known as a critical mediator in allergy and asthma.³⁰ Its role in cardiovascular diseases (CVDs) has been evaluated in recent studies. The prolonged IL-13 synthesis appears to be a risk factor for adverse outcomes chronic CVDs, such as heart failure, largely due to the induction of fibrosis and adverse cardiac remodeling.³¹ The liberation of TGF- β , IL-4, together with IL-13 elevation following massive cell death after radiotherapy, plays critical roles in the regulating ECM deposition and fibroblast activation. These cytokines can also trigger continuous ROS production.³²

Our results confirm that daunorubicin induces inflammation, as the daunorubicin group (Group II) showed a significant elevation in serum levels of NF- κ B and cardiac tissue levels of pro-inflammatory mediators TNF- α and IL-13.

The results of our study also show co-administration of omega-3 with daunorubicin (Group IV) significantly reduced the serum levels of NF- κ B and cardiac tissue levels of TNF- α and IL-13, suggesting suppression of inflammatory signaling, as demonstrated by the other studies.^{6,7}

Furthermore, the present study, for the first time, reports the anti-inflammatory effect of TND1128, as shown by a significant reduction in the serum NF- κ B levels and cardiac tissue levels of TNF- α and IL-13 in the TND1128+daunorubicin group (Group VI), suggesting suppression of inflammatory signaling.

C-reactive protein (CRP), an acute-phase protein, primary regulated by IL-1 β , TNF- α and IL-6. Serum levels of TNF- α , IL-6, and CRP have been reported to be elevated in congestive heart failure patients, regardless of etiology.³³ CRP correlates with an increased risk of myocardial infarction, cardiovascular disease, and stroke among apparently healthy individuals. CRP induces apoptosis mediated by caspases of human coronary vascular smooth muscle cells (VSMCs).³⁴

In the intrinsic apoptotic pathway, Bax, a pro-apoptotic protein in mitochondria, causes the liberation of cytochrome C, endoribonuclease G, and other mitochondrial proteins through the opening of the outer membrane of the mitochondria; this release is mediated by the permeability transition pore of the mitochondria. This release of cytochrome C leads to the activation of caspase-9 and subsequently caspase-3, resulting in apoptosis.²⁸ It is well established that the capacity of different chemotherapeutic drugs to induce apoptosis is correlated with their anticancer efficacy.³⁵ The present study revealed a considerable elevation in cardiac tissue levels of cleaved caspase-3 in the daunorubicin group (Group II). This aligns with previous findings that anthracyclines, including daunorubicin, induce apoptosis in cardiomyocytes.^{3,36} Furthermore, caspase-3 levels, as measured by Western blot, were significantly lowered in

the omega-3 + daunorubicin group (Group IV), indicating effective mitigation of apoptotic processes, as observed by other study.³⁷

TND1128 has been reported to improve the morphology of neurons and facilitate polarization of mitochondrial membrane potential and the ATP production in brain slices.²⁷ While TND1128 has not previously been described as an apoptosis, the present study, for the first time demonstrates its ability to effectively inhibit apoptotic processes, as shown by substantial reduction in caspase-3 levels in rats treated with TND1128+daunorubicin (Group IV). The ability of TND1128 to stimulate mitochondrial function and exert antioxidant effects may indirectly contribute to the cell survival and potentially counteract the apoptosis.

Histological examination of cardiac tissue further substantiated the biochemical findings. The control group (Group I) showed normal myocardial architecture, while the daunorubicin-treated group (Group II) displayed extensive myocardial fiber disintegration, edema, vascular congestion, and inflammatory infiltration hallmarks of drug-induced cardiomyopathy, as reported by other studies.³⁸ Cardiac tissue sections from the co-administration of omega-3 with daunorubicin group (Group IV) exhibited considerable improvement in tissue morphology with minimal necrosis, suggesting morphological preservation, consistent with other studies.³⁹

Cardiac tissue sections from the co-administration of TND1128 with daunorubicin (Group VI) exhibited largely preserved myocardial structure; however, some areas showed focal hemorrhages and an increase in perimysial connective tissue. These findings demonstrate the cytoprotective effect of TND1128, likely mediated through mitochondrial activation and preservation of cardiac tissue architecture—a phenomenon not previously described but observed in this study (Figure 9F).

Conclusion

This study offers compelling confirmation for the cardioprotective potential of both omega-3 fatty acids and TND1128, a novel 5-deazaflavin derivative, against daunorubicin-induced cardiotoxicity in a rat model. The study employed an extensive multi-parametric approach, including biochemical markers (troponin I, CK-MB, MDA, GSH, NF- κ B), gene expression profiles (TNF- α , IL-13), Western blotting (cleaved caspase-3), and histopathological evaluation in Wistar rats, in which administration of daunorubicin resulted in significant oxidative stress, inflammation, apoptosis, and structural damage to myocardial tissues. Both omega-3 and TND1128 were able to alleviate these deleterious changes to a considerable extent.

Notably, these results of our study highlighted the mechanisms of TND1128 that had not been previously described, including the anti-inflammatory and anti-apoptotic pathways as well as preservation of cardiac

tissue architecture. These findings position TND1128 as a potential therapeutic agent in combating anthracycline-induced cardiotoxicity. While the study provides strong preclinical data in a rat model, extending these findings for individuals remains challenging. TND1128's pharmacokinetic profile, metabolic stability, and possible toxicity in humans have not been investigated. More pharmacological and toxicological studies are required before considering its possible clinical use.

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Authors' Contribution

Conceptualization: Alaa Radhi Khudhair.

Formal analysis: Huda Hameed Rasheed.

Investigation: Huda Hameed Rasheed.

Writing—original draft: Huda Hameed Rasheed, Alaa Radhi Khudhair.

Competing Interests

There is no conflict of interest regarding the publication of my manuscript. Funding The research did not receive financial support from an Institution.

Ethical Approval

The Research was approved by the Ethical Committee of the Department of Pharmacology and Toxicology, College of Pharmacy/ University of Baghdad before the start of the study.

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