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Full-length Research Article

Therapeutic Potential of Tadalafil in Doxorubicin-Induced Pulmonary and Haematological Toxicities in Wistar Rats

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Summary: Doxorubicin (DOX) therapy is associated with pulmonary toxicity and hematotoxicity as its off-target side effects. In this study, tadalafil (TAD) was investigated for its chemopreventive potential against DOX-induced pulmonary toxicity and hematotoxicity in 48 male Wistar rats that were divided into 8 groups of 6 rats/group and orally pretreated with 2.5 mg/kg/day, 5.0 mg/kg/day, and 10 mg/kg/day TAD 1 hour before intraperitoneal injection of 2.5 mg/kg DOX on alternate days for 12 days after which the rats were humanely sacrificed. Blood samples for haematological and biochemical endpoints, and lung tissue samples for oxidative stress markers, pro-inflammatory cytokine assays, and histopathology were collected. The rats' body weights were measured at the start and end of the experiments. Results showed that DOX toxicity was associated with significant ($p<0.0001$) weight loss, with corresponding significant ($p<0.05$) reduction in the relative lung weight. DOX intoxication was also associated with leukopenia, thrombocytopenia, lymphocytopenia, myelocytosis, and neutrophilia, suggesting bone marrow suppression, while it induced significant ($p<0.0001$) decreases in the serum bicarbonate and pH levels, as well as an increase in iCa^{2+} levels. DOX intoxication was also associated with profound ($p<0.0001$) increases in the lung tissue oxidative stress markers. However, oral TAD pretreatment did not significantly ($p>0.05$) improve DOX-associated weight loss but reversed the decrease in relative lung weight. TAD pretreatments also profoundly ($p<0.001$, $p<0.0001$) reversed both the DOX-induced haematological and biochemical alterations. Overall, TAD may have potential protective effects on DOX-induced pulmonary and haematological dysfunctions, highlighting the chemopreventive potential of TAD, which was probably mediated via antioxidant and/or free radical scavenging and anti-inflammatory mechanisms.

Keywords: Doxorubicin, pulmonary function biomarkers, complete blood count, pro-inflammatory cytokines, oxidative stress markers, Tadalafil.

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INTRODUCTION

Doxorubicin (DOX), a broad-spectrum anthracycline cytotoxic antibiotic, is an effective and potent antitumoral drug used for the treatment of diverse hematological and solid tumors such as soft and solid tissue tumors such as the sarcomas, cancers of the breast, ovary, bladder and the thyroid, lymphoblastic leukemia, acute myeloblastic leukemia, Hodgkin lymphoma, and small cell lung cancer (Qi *et al.*, 2020; Johnson-Arbor *et al.*, 2024; Kudelkina *et al.*, 2025). DOX's precise mechanisms of cytotoxic action are complex and debatable. However, there is a consensus in the scientific community that DOX's cytotoxic mechanisms include inhibition of cell proliferation, oxidative stress induction, free radical generation, inhibition of RNA and DNA synthesis via topoisomerase II enzyme inhibition, induction of inflammation, cell death acceleration mainly via the induction of autophagy and apoptosis (Jabłońska-Trypuć *et al.*, 2018; Rawat *et al.*, 2021; Zhao *et al.*, 2023; Wang *et al.*, 2024). Its clinical use, however, is limited by the multi-organ off-target side effects that it causes, resulting from its multi-directional cytotoxic effects and non-specificity to cancerous cells (Xing *et al.*, 2022). The DOX-mediated multi-organ toxicities include cardiotoxicity, hepatotoxicity, nephrotoxicity, pulmonary toxicity, myelosuppression, and gonadotoxicity (Qi *et al.*, 2020; Zhao *et al.*, 2023).

Pulmonary toxicity and hematotoxicity are well-recognised and documented off-target side effects of prolonged DOX chemotherapy (Vershoore *et al.*, 1987;

Eisenbens *et al.*, 2001; Nevadunsky *et al.*, 2013). These off-target side effects have limited the clinical use of DOX in human cancer therapy (Guzel *et al.*, 2021).

Although the precise causative role of DOX in pulmonary toxicity remains poorly understood, oxidative stress signalling remains the foremost etiopathogenetic mechanism of this toxicity (Skeoch *et al.*, 2018). Oxidative stress induces intracellular accumulation of reactive oxygen species (ROS), including superoxides that can cause lipid peroxidation and injury to proteins and DNA (Qi *et al.*, 2020; Mazzotta *et al.*, 2016). More so, DOX is known to significantly decrease the endogenous antioxidant profile, further destabilizing the antioxidant/oxidant homeostasis (Guzel *et al.*, 2021). Cumulatively, these result in the disturbances of pulmonary tissue and bone marrow antioxidant/oxidant homeostasis to cause pulmonary toxicity and hematotoxicity (Jablońska-Trypuć *et al.*, 2018; Guzel and Tekremur, 2021). Given the above, managing ROS accumulation and maintaining antioxidant/oxidant balance homeostasis is a potentially critical therapeutic strategy for DOX-induced pulmonary toxicity and hematotoxicity (Guzel and Tekremur, 2021).

Tadalafil (TAD) is a selective, long-acting phosphodiesterase type 5 inhibitor that is widely known for its beneficial use in the clinical management of erectile dysfunction (ED) and pulmonary artery hypertension (PAH) (Klinger, 2011; Duvanti et al., 2017; Lee et al., 2023). TAD is also documented to be an effective antioxidant (Duvanti *et al.*, 2017; Sheweita *et al.*, 2020; Yeni *et al.*, 2022).
Despite being recognized for over a few decades, there are little or no therapeutic remedies available to either prevent the onset or treat these off-target side effects when they arise in the course of DOX therapy. Thus, this is the basis for this current study, which evaluates the TAD's ameliorating potential against DOX-induced pulmonary and haematological toxicities in young adult male Wistar rats.

MATERIALS AND METHODS

Care and Use of experimental rats: Adult male Wistar Albino rats (aged 10-12 weeks old and body weight: 180-200 g) were procured from Bayo Farms, Sango-Ota, Ogun State, after obtaining an institutional ethical approval (with the reference number: LASU/23/REC/083) from the Lagos State University Research Ethics Committee (LASU-REC), Main Campus, Ojo, Lagos State, Nigeria. The rats were acclimatized in the Lagos State University College of Medicine (LASUCOM) Animal House for two (2) weeks. The rats were cared for and handled in line with global best practices guiding the Use and Handling of Experimental Animals as stipulated by the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011). Standard rat chow and potable tap water were made freely available for the rats and maintained at standard laboratory conditions throughout the study.

Body Weight Measurement: A rodent digital weighing scale (Model: Virgo Electronic Compact Scale®, New Delhi, India) was used to take the rat body weights at the beginning and end of the study. The rat weights obtained were in grams (g).

Drug pre-treatment and experimental induction of DOX-induced pulmonary and haematological toxicities in the treated rats: Pulmonary and haematological toxicities were induced with DOX using the method earlier described by Adeneye *et al.* (2021). Briefly described, the experimental rats were randomly allocated into eight (8) groups of six (6) rats per group, such that the weight variations within and between groups do not exceed ± 20% of the average weights of the sample population.

The drug treatment of each group is in Table 1. The experimental rats were pretreated with oral sterile water, silymarin (Silybon-140®, Micro Labs Limited, 92 Sipcot Hosur-635126, India), and tadalafil (Honnonil®, Lyn-Edge Pharmaceutical Limited, Chevron Alternative Route, Poroku, Lekki, Lagos State, Nigeria) one hour before administering 2.5 mg/kg DOX (Oncodox-50®, Cipla Limited, Plot No. 5, S-103 Verna, Goa403-722, India) given intraperitoneally. The choice of drug doses used was based on the results of the preliminary studies and the literature searches.

Table 1.
Experimental Groups and Respective Treatment Protocols

Groups	Treatment Protocols
Group I	10 ml/kg/day of sterile water given *p.o.* daily + 1 ml/kg/day of sterile water given *i.p.* on alternate days for 12 days
Group II	10 ml/kg/day sterile water given *p.o.* daily + 2.5 mg/kg/day *DOX* in sterile water given *i.p.* on alternate days for 12 days
Group III	20 mg/kg/day silymarin in sterile water given *p.o.* daily + 1 ml/kg/day sterile water *i.p.* on alternate days for 12 days
Group IV	20 mg/kg/day silymarin in sterile water given *p.o.* daily + 2.5 mg/kg/day *DOX* in sterile water given *i.p.* on alternate days for 12 days
Group V	5 mg/kg/day tadalafil in sterile water given *p.o.* daily + 1 ml/kg/day sterile water given *i.p.* on alternate days for 12 days
Group VI	2.5 mg/kg/day tadalafil in sterile water given *p.o.* daily + 2.5 mg/kg/day *DOX* in sterile water given *i.p.* on alternate days for 12 days
Group VII	5 mg/kg/day tadalafil in sterile water given *p.o.* daily + 2.5 mg/kg/day *DOX* in sterile water given *i.p.* on alternate days for 12 days
Group VIII	10 mg/kg/day tadalafil in sterile water given *p.o.* + 2.5 mg/kg/day *DOX* in sterile water given *i.p.* on alternate days for 12 days

Blood Samples and Tissues Collection: Twenty-four (24) hours after the last DOX injection on day 12 of the treatment, treated rats were fasted overnight and humanely sacrificed under light inhaled halothane anesthesia for whole blood sample collection directly from the heart with fine 21G hypodermic needle and 5 ml syringe without causing damage to the heart tissues. A long surgical incision was made on the ventral surface of the thorax and abdomen and gently retracted to expose the abdominal organs. The liver and kidneys were identified, carefully dissected en bloc, and weighed on a digital weighing balance with the weight values expressed in grams (g).
At the end of the experiment, the rat carcasses were evacuated and duly processed by the trained and certified Animal House Attendants.

Calculation of percentage weight changes (%Δwt): The percentage weight change (%Δwt) was calculated as a ratio of the difference between the final and initial body weights and the initial body weight multiplied by 100 which is expressed mathematically in the equation:

$$\{[final\ body\ weight\ (g) - initial\ body\ weight\ (g)] / [initial\ body\ weight\ (g)]\} \times 100$$

Calculation of relative liver and kidney weights

The respective relative kidney weight was calculated as the ratio of the absolute weight (g) of both kidneys and the final rat body weight (g) multiplied by 100. This is expressed mathematically as:

$$\{[absolute\ organ\ weight\ (g)] / [final\ rat\ weight]\}\ x\ 100$$

Blood sample collection and determination of pulmonary function test: For each rat, 4-5 ml of whole blood was collected directly from the heart chamber using a 21G needle mounted on a 5 ml syringe. 2 ml of the fresh blood sample was collected into EDTA-treated sample blood and placed on auto stirrer to prevent the blood from clotting was used for the complete blood count test. The remaining blood was collected into a plain sample bottle and allowed to stand at 4ºC for 4 hours before being centrifuged at 5000 revolutions/min for 5 minutes to separate the sera from the other clotted blood components. Then, the serum was separated and used for serum pH, bicarbonate (HCO_3^-), and ionized calcium (Ca^{2+}) estimation. The bio-assays were carried out using standard analytical procedures and the Manufacturer's instructions on the enclosed leaflets in the commercial diagnostic test kits (Randox Diagnostics Test Kits®, Randox Laboratories Ltd, Crumlin, United Kingdom).

Lung tissue antioxidant enzymatic assays and pro inflammatory markers: After the rats were sacrificed humanely under inhaled light halothane anesthesia, the lungs were dissected en bloc and rinsed briskly in ice-cold 1.15% KCl solution to preserve the tissue's oxidative enzyme activities before being kept in a clean sample bottle that was in an ice-pack-filled cooler. The tissues were homogenized using a mechanical homogenizer with a Teflon ice-cold sodium phosphate buffer (0.1M, pH 7.4). The tissue homogenates were centrifuged at 5000 revolutions per minute for 10 min at 4˚C. The supernatants were used for the determination of activities of the tissue oxidative stress markers (GSH, MDA, CAT, SOD, GST, and GPx) as described by Olorundare *et al.* (2020, 2021). The antioxidant enzyme activities were expressed as U/ mg protein.

The pro-inflammatory markers: interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) levels in the rat lung tissues were also determined using rat-specific commercial ELISA kits sourced from ElabScience (14780 Memorial Drive, Suite 105, Houston, Texas, 77079, USA).

Histopathological studies of the lung tissues: The remaining one-half of the dissected lung tissues was preserved in 10% formo-saline solution. The tissue slide preparation and reading were done using the procedures earlier described by Olorundare *et al*. (2020).

Statistical Analysis of Data

The average body weight, body weight changes, and biochemical assays were expressed as mean ± standard deviation (S.D.) and mean ± standard error of the mean (S.E.M.) of six observations, respectively. The statistical analysis using a One-way analysis of variance followed by Tukey's post hoc test on GraphPad Prism version 5 was adopted. The levels of statistical significance were at $p<0.05$, $p<0.001$, and $p<0.0001$.

RESULTS

Modulatory impact of TAD pre-treatment on DOX-induced alterations in body weight dynamics in rats: Table 2 shows that alternate-day intraperitoneal injections with DOX at 2.5 mg/kg for 12 days caused the most significant ($p<0.0001$) weight losses in the final average body weight and percentage body weight changes (137.40 ± 20.68g, and -28.22 ± 04.22%, respectively) in the DOX-only treated (Group II) rats when compared with the untreated control (Group I) rats (182.50 ± 22.60 g, and 05.05 ± 03.85%, respectively) (Table 2).

Daily oral pretreatments with SIL (used as a standard antioxidant drug) and graded TAD (2.5 mg/kg body weight/day, 5 mg/kg body weight/day, and 10 mg/kg body weight/day) were also associated with losses in the final average body weight and percentage body weight changes (143.10 ± 25.71 g and -16.37 ± 22.07%; 158.50 ± 24.75 g and -22.78 ± 06.82%; 154.00 ± 26.05 g and -21.63 ± 06.61%; 150.10 ± 17.88 g and -25.00 ± 03.22%, respectively) with the most significant further weight losses recorded in the 10 mg/kg body weight/day TAD pretreated, DOX intoxicated rats (Table 2).

Table 2. Effects of DOX intoxication, and graded oral TAD pre-treatment on the mean initial body weights (initial Wt.), mean final body weight (final Wt.) and percentage body weight changes (%ΔWt) in treated rats.

Groups	initial Wt. (g)	final Wt. (g)	%ΔWt
I	173.0 ± 21.2	182.5 ± 22.6	05.1 ± 03.9
II	192.2 ± 16.6	137.4 ± 20.7^{c}	-28.2 ± 04.2^{c-}
III	193.7 ± 05.9	207.2 ± 12.1$^{a+, a\#}$	07.10 ± 06.08$^{c\#}$
IV	186.8 ± 05.6	143.1 ± 25.7^{c}	16.37 ± 22.02^{c-}
V	200.7 ± 22.8	218.8 ± 18.6$^{a+, c\#}$	05.80 ± 4.41$^{c\#}$
VI	204.7 ± 21.6	158.5 ± 24.8^{c-}	22.78 ± 06.82^{c}
VII	195.5 ± 19.5	154.0 ± 26.1^{b-}	21.63 ± 06.61^{c-}
VIII	199.7 ± 20.2	150.1 ± 17.9^{c-}	25.00 ± 03.22^{c-}

$^{b-}$ and $^{c-}$ represent significant decreases at $p<0.001$ and $p<0.0001$, respectively, when compared to untreated normal control (Group I) values while $^{a+}$ and $^{c+}$ represent significant increases at $p<0.05$ and $p<0.0001$, respectively, when compared to untreated normal control (Group I) values; $^{a\#}$ and $^{c\#}$ represent significant increases at $p<0.05$ and $p<0.0001$, respectively, when compared to untreated DOX intoxicated (Group II) values.

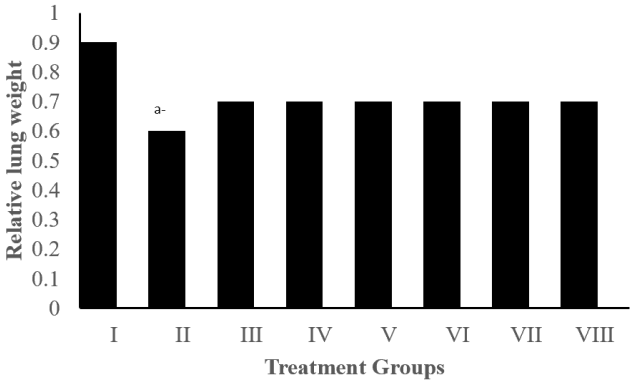

Figure 1.
Effect of DOX intoxication and graded tadalafil (TAD) and silymarin (SIL) pretreatments on the relative lung weight of treated rats
[a-] represents a significant decrease at p<0.005 when compared to the untreated normal control (Group I) value.

TAD ameliorating influence on doxorubicin-induced pulmonary weight alterations in rats: On the relative lung weight, DOX intoxication caused a significant decrease ($p<0.05$) in the relative lung weight (0.6 ± 0.1) (Figure 1). However, oral SIL and TAD pre-treatments significantly ($p<0.05$) reversed the DOX-induced reduction in the relative lung weight and restored it to control values (0.7 ± 0.1, 0.7 ± 0.1, 0.7 ± 0.1, and 0.7 ± 0.1, respectively) (Figure 1).

Tadalafil's ameliorative effects on doxorubicin-altered pulmonary function parameters: A focus on acid-base and calcium homeostasis in treated rats: Repeated intraperitoneal injections of 2.5 mg/kg body weight DOX resulted in significant ($p<0.001$, $p<0.0001$) decreases in the serum bicarbonate (HCO_3^-) and pH levels when compared to untreated control (Group I) values (Table 3). DOX intoxication also caused a significant ($p<0.05$) increase in the ionized calcium (iCa^{2+}) level when compared to untreated control (Group I) values (Table 3).

Oral SIL and TAD pretreatments resulted in significant increases ($p<0.05$, $p<0.001$, and $p<0.0001$) in the serum HCO3- and pH levels when compared with the values obtained for untreated DOX intoxicated (Group II) rats (Table 3). Conversely, oral SIL and TAD pre-treatment significantly ($p<0.05$ and $p<0.001$) increased the circulating iCa^{2+} when compared to the untreated control (Group I) values but not significantly ($p>0.05$) different from those of untreated DOX intoxicated (Group II) rats (Table 3).

Table 3.
Effects of DOX intoxication, and graded oral TAD pre-treatment on the lung function parameters (HCO_3^- and pH) and ionized calcium ion (iCa^{2+}) in treated rats

Groups	HCO_3 (mmol/L)	iCa^{2+} (mmol/L)	pH
I	23.7 ± 0.9	1.2 ± 0.0	7.5 ± 0.0
II	$18.4 \pm 2.4^{b-}$	$1.6 \pm 0.1^{a+}$	$7.4 \pm 0.1^{a-}$
III	$25.0 \pm 0.6^{b\#}$	$1.2 \pm 0.0^{a*}$	7.5 ± 0.0
IV	$23.5 \pm 1.5^{b\#}$	$1.2 \pm 0.0^{a*}$	$7.6 \pm 0.1^{a\#}$
V	$28.1 \pm 0.8^{b\#}$	$1.2 \pm 0.0^{a*}$	7.5 ± 0.0
VI	20.7±1.9	$1.2±0.1^{a*}$	7.5±0.0
VII	$23.0±1.7^{b\#}$	$1.3±0.1^{a*}$	$7.6±0.1^{b*}$
VIII	$25.6±1.0^{b\#}$	$1.3±0.0^{a*}$	$7.6±0.0^{b*}$

[a+] represents a significant increase at p<0.05 while [a-] and [b-] represent significant decreases at p<0.05 and p<0.001, respectively, when compared to untreated normal control (Group I) values. [a] and [b*] represent significant decreases at p<0.05 and p<0.001, respectively, while [a#] and [b#] represent significant increases at p<0.05 and p<0.001, respectively, when compared to untreated DOX intoxicated control (Group II) values*

Navigating the oxidative storm: TAD's therapeutic compass guiding pulmonary antioxidant defenses against DOX-induced imbalance in treated rats: The effects of DOX intoxication and oral SIL and TAD pre-treatments on the lung tissue antioxidant profile are in Table 4. Repeated alternate-day treatments with intraperitoneal injections of 2.5 mg/kg body weight DOX for 12 days resulted in a significant ($p<0.0001$) decrease in the lung tissue SOD, CAT, GST, and GPx activities and a remarkable ($p<0.0001$) increase and decrease in the pulmonary tissue MDA and GSH levels, respectively when compared to untreated control (Group I) values (Table 4). However, oral with SIL and graded TAD pre-treatments, there were significant increases and reversal ($p<0.001$ and $p<0.0001$) in the lung tissue SOD, GST, and GPx activities, restoring their activities to the control values but caused no significant ($p<0.05$) reversal in the CAT activities of the DOX-intoxicated lung tissues (Table 4). Similarly, oral SIL and TAD pre-treatments also significantly ($p<0.0001$) increased the lung tissue GSH levels when compared to the untreated DOX-intoxicated (Group II) values (Table 4). On the lung tissue MDA activities, oral SIL and TAD pre-treatments also significantly ($p<0.001$, $p<0.0001$) decreased the MDA activities in the DOX-intoxicated lung tissues (Table 4).

Table 4.
Effects of DOX intoxication, and graded oral TAD pre-treatment on the lung antioxidant profile in treated rats

Groups	SOD	CAT	MD	GST	GPx	GSH
I	3.7±0.3	21.3±0.6	1.6±0.0	25.9±2.0	53.8±1.3	39.3±1.0
II	$1.7±0.1^{c-}$	$18.2±0.3^{c-}$	$4.5±0.2^{c+}$	$20.6±0.5^{a-}$	$31.4±0.6^{c-}$	$26.3±1.1^{c-}$
III	$4.6±0.3^{c\#}$	$25.4±1.8^{c\#}$	$1.7±0.2^{c-}$	$36.2±1.7^{b\#}$	$55.4±2.1^{c\#}$	$40.9±1.4^{c\#}$
IV	$5.7±0.3^{c\#}$	19.5±0.5	$2.4±0.1^{b*}$	$46.6±1.8^{c\#}$	$67.7±0.6^{c\#}$	$50.4±0.5^{c\#}$
V	$5.4±0.3^{c\#}$	19.1±0.3	$2.4±0.0^{b*}$	$49.6±1.6^{b\#}$	$70.4±1.0^{c\#}$	$54.2±0.7^{c\#}$
VI	$4.8±0.2^{c\#}$	18.7±0.2	$2.6±0.0^{b*}$	$48.4±1.7^{b\#}$	$61.5±11.0^{c\#}$	$55.0±1.0^{c\#}$
VII	$4.4±0.3^{c\#}$	18.4±0.2	$2.7±0.0^{b*}$	$46.9±2.2^{c\#}$	$71.2±1.7^{c\#}$	$54.0±0.9^{c\#}$
VIII	$5.0±0.1^{c\#}$	17.1±0.5	$2.6±0.0^{c*}$	$50.2±1.0^{c\#}$	$69.7±1.2^{c\#}$	$55.4±1.0^{c\#}$

[c+] represents a significant increase at p<0.0001 while [a-] and [c-] represent significant decreases at p<0.05 and p<0.0001, respectively, when compared to untreated normal (Group I) rats. [b] and [c*] represent significant decreases at p<0.001 and* p<0.0001, respectively, while [b#] and [c#] represent significant increases at p<0.001 and p<0.0001, respectively, when compared to untreated DOX intoxicated (Group II) rats.

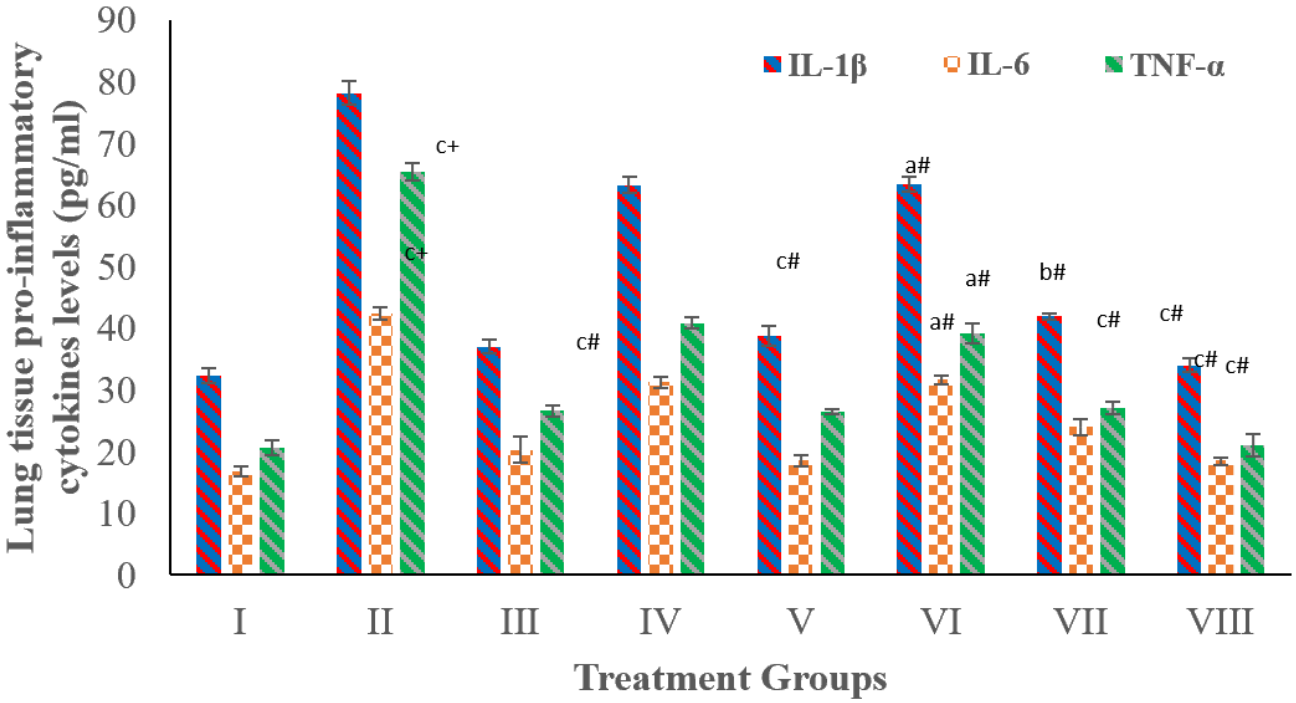

Figure 2.
Effect of graded tadalafil (TAD) and silymarin (SIL) on the lung tissue pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) levels of doxorubicin(DOX)-intoxicated control (Group II) values
$^{c+}$represents a significant increase at $p<0.0001$ when compared to the untreated normal control (Group I) values while $^{a\#, b\#}$ and $^{c\#}$ represent significant decreases at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, when compared to untreated DOX intoxicated (Group II) values.

Pro-inflammatory imprint (IL-1, IL-6 and TNF-α) of DOX and the mitigating artistry of TAD on the treated rat lung tissues: In the untreated DOX intoxicated group, there were significant ($p<0.001$ and $p<0.0001$) increases in the lung tissue levels of the measured pro-inflammatory cytokines: IL-1β, IL-6, and TNF-α levels were recorded compared to the untreated normal rats (Figure 2). However, TAD and SIL pre-treatments caused significant ($p<0.05$, $p<0.001$, and $p<0.0001$) decreases in lung tissue concentrations of IL-1β, IL-6, and TNF-α and reverting the values to almost untreated control values when compared to untreated DOX intoxicated group (Figure 2).

Hematological effect of DOX intoxication and ameliorating effects of TAD pre-treatments in rats: Table 5a shows the effects of DOX intoxication and the oral SIL and TAD pre-treatments on the hemogram of the treated rats consisting of the red blood cell counts (RBC), hemoglobin concentration (Hb), packed cell volume or hematocrit (PCV), mean corpuscular hemoglobin concentration (MCHC), total white blood cell counts (TWBC) and platelets count (PLT). Repeated i.p. injections of 2.5 mg/kg body weight DOX on alternate days for 12 days resulted in significant ($p<0.001$) decreases in TWBC and PLT without any significant ($p>0.05$) effect on RBC, Hb, PCV, MCV, and MCHC indices (Table 5a). With SIL and graded TAD pre-treatments, there were significant ($p<0.05$, $p<0.001$, $p<0.0001$) improvements in the TWBC and PLT indices while causing non-significant ($p>0.05$) alterations in the RBC, Hb, PCV, and MCHC values (Table 5a).

DOX intoxication also resulted in a significant ($p<0.0001$) decrease and increase in %LYMP and %NEUT, respectively, when compared to the untreated control (Group I) values but caused non-significant ($p>0.05$) alterations in the %MXD value (Table 5b). However, SIL and graded TAD pre-treatments significantly ($p<0.001$ and $p<0.0001$) reversed these changes and restored these hematological indices to about control values (Table 5b).

Histopathological effect of TAD pre-treatments on DOX-intoxicated rat lung tissues: Repeated DOX injection to the treated rat liver was associated with severe pulmonary interstitial congestion with cellular infiltration, diffuse inter-alveolar septa thickening, and diffuse alveolar collapse (Figure 3b) when compared to the untreated normal pulmonary histoarchitecture (Figure 3a).
Oral pre-treatment with SIL in normal rats was not associated with any remarkable pulmonary lesions (Figure 3c). SIL pre-treatment in DOX-intoxicated rats was associated with moderate pulmonary interstitial congestion and focal bronchiolar congestion with cellular infiltration (Figure 3d). In normal rats pretreated with 5 mg/kg/day TAD, there were no remarkable histological changes in the bronchiolo-alveolar architecture and pulmonary interstitium of treated rats (Figure 3e). However, oral pretreatment with graded TAD doses at 2.5 mg/kg/day, 5 mg/kg/day, and 10 mg/kg/day showed mild-to-moderate pulmonary interstitial congestion and focal alveolar collapse (Figure 3f); mild pulmonary interstitial congestion with widening of alveoli (Figure 3g), and very mild pulmonary interstitial congestion and normal alveolar distribution (Figure 3h), respectively.

Table 5a.
Effects of DOX intoxication, and graded oral TAD pre-treatment on the hematological parameters (RBC, Hb, PCV, MCHC, TWBC and PLT) in treated rats

Groups	RBC ($x10^6$/L)	Hb (g/dL)	PCV (%)	MCHC (g/dL)	TWBC ($x10^3$/L)	PLT ($x10^3$/µL)
I	7.7±0.4	12.9±0.5	46.1±2.1	31.7±1.0	11.8±1.2	653.8±99.9
II	7.6±0.7	13.2±1.1	45.2±3.5	31.5±0.1	$1.6±0.2^{c-}$	$190.0±14.8^{c-}$
III	7.7±0.2	13.8±0.4	48.2±3.5	30.6±0.2	13.1±1.7	$979.5±164.0^{c\#}$
IV	7.3±0.3	12.9±0.2	41.7±1.0	31.0±0.5	$4.1±1.6a^{\#}$	$678.7±124.2^{b\#}$
V	7.8±0.2	14.0±0.3	44.9±1.1	31.2±0.3	$13.3±1.6^{a\#}$	$856.7±225.7^{c\#}$
VI	7.4±0.5	13.1±0.8	42.0±2.9	31.3±0.5	$3.1±0.6^{a\#}$	$186.2±138.2^{c*}$
VII	7.8±0.8	12.8±1.1	39.7±1.0	37.2±3.2	$5.0±1.2^{a\#}$	$414.5±98.0^{b\#}$
VIII	7.4±0.5	13.2±0.7	41.6±2.3	31.8±0.5	$6.7±1.2^{b\#}$	$627.3±133.4^{c\#}$

$^{c-}$ represents a significant decrease at $p<0.0001$ when compared to untreated normal control (Group I) values. ^{c*} represents a significant decrease at $p<0.0001$ while $^{a\#, b\#}$ and $^{c\#}$ represent significant increases at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively, when compared to untreated DOX intoxicated control (Group II) values

Table 5b.
Effects of DOX intoxication, oral SIL and graded oral TAD pre-treatment on the white blood cell differentials {percentage lymphocytes count (%LYMP), percentage neutrophil counts (%NEUT) and percentage myelocyte counts (%MXD) in treated rats

Groups	%LYMP	%NEUT	%MXD
I	81.4 ± 3.8	11.2 ± 2.1	4.0 ± 0.7
II	$37.9 ± 0.70^{c-}$	$59.4 ± 1.0^{c+}$	3.3 ± 0.3
III	$76.3 ± 6.4^{c\#}$	$19.6 ± 5.6^{c*}$	4.0 ± 0.8
IV	$59.1 ± 11.8^{b\#}$	$38.1 ± 11.4^{b*}$	2.8 ± 0.9
V	$74.4 ± 4.0^{c\#}$	$21.4 ± 3.5^{b*}$	4.2 ± 0.6
VI	$55.4±10.6^{b\#}$	$31.6±7.6^{b*}$	$13.1±9.8^{b+,\#}$
VII	$27.7±11.8^{c*}$	$68.9.3±11.7^{c\#}$	$13.5±0.6^{b+,\#}$
VIII	$47.0±5.5^{a\#}$	$39.4±6.2^{b*}$	$19.1±10.1^{c+,\#}$

$^{a+}$ represents a significant increase at $p<0.05$ while $^{a-}$ and $^{b-}$ represent significant decreases at $p<0.05$ and $p<0.001$, respectively, when compared to untreated normal control (Group I) values. ^{a*} and ^{b*} represent significant decreases at $p<0.05$ and $p<0.001$, respectively, while $^{a\#}$ and $^{b\#}$ represent significant increases at $p<0.05$ and $p<0.001$, respectively, when compared to untreated DOX-intoxicated control (Group II) values

DISCUSSION

DOX is commonly used to treat various types of cancers, including breast cancer, leukemia, and lymphoma. One of the side effects of this drug is weight loss, which can be attributed to anorexia and muscle wasting (Hiensch *et al.*, 2020; Pedrosa *et al.*, 2023). Also, DOX is known to cause gastrointestinal side effects such as nausea, vomiting, and diarrhea, which can lead to decreased food intake and subsequent weight loss. Additionally, DOX can cause taste changes that affect appetite and cause anorexia. In the present study, treatment with 15 mg/kg DOX (2.5 mg/kg given on alternate days for 12 days) caused diarrhea from day 8 of the drug treatment and lent support to the previous reports of DOX-induced diarrhea resulting from either enterocolitis or intestinal mucositis (Dahlgren *et al.*, 2021; Kawasaki *et al.*, 2023). However, the diarrhea observed in this study appears to be more like neutropenic enterocolitis (also referred to as "typhlitis" or "necrotizing enteropathy") based on the fact that this is the type of enterocolitis that is often associated with patients with DOX-induced neutropenia (Cherri *et al.*, 2020). Neutropenic enterocolitis (NEC) is a necrotizing inflammatory condition majorly affecting the terminal portion of the small intestine (often the terminal ileum) and the upper large intestine (cecum and ascending colon) in the face of neutropenia (Nesher and Rolston, 2013; Sirakaya and Inanç, 2020; Babakhanlou *et al.*, 2023). The fact that the differential leukocyte counts in the study revealed the presence of neutropenia and diarrhea affirms the induction of neutropenic enterocolitis associated with anorexia from day 10 of the DOX treatment. However, the fact that daily oral pretreatments with SIL and graded TAD did not improve the DOX-associated weight loss suggested that these drugs were not effective attenuators of this side-effect of prolonged DOX therapy. Conversely, the fact that oral SIL and TAD pretreatments reversed the profound decrease in the relative lung weight was also significant and still indicated the protective effect of these drugs on the DOX-treated rat lungs.

Pulmonary toxicity and hematotoxicity are life-threatening off-target side effects of long-term DOX therapy that are well documented in the literature (Minchin *et al.*, 1988; Testart-Pailler *et al.*, 2007; Kameo *et al.*, 2012; Kanno and Hara, 2021; Allan *et al.*, 2021; Lustberg *et al.*, 2023). DOX-induced hematotoxicity may manifest as myelosuppression, anemia, leukopenia, neutropenia, and thrombocytopenia (Thorn *et al.*, 2011; Kameo *et al.*, 2012; Madeddu *et al.*, 2021). In this study, repeated DOX administration for 12 days was associated with leukopenia, thrombocytopenia, lymphocytopenia, and neutrophilia, and these are considered predictors of DOX-induced hematological toxicity (Sleijfer *et al.*, 2018). Thus, the presence of the predictors in the hematological results obtained in this study suggests the establishment of doxorubicin toxicity. Neutrophilia is often considered a strong indicator of inflammation and cancers (Rosales, 2018; Zahorec, 2021). However, the former appears to be the more likely case, as healthy and non-cancerous rats were studied. The fact that neutrophilia was in the clinical blood picture of the DOX-treated rats suggests that DOX could be mediating its hematotoxicity via inflammation, a well-documented off-target toxicity mechanism for DOX (Supriya et al., 2016; Reis-Mendes *et al.*, 2021; 2023). This hypothesis was, perhaps, corroborated by the remarkable increases in the levels of pulmonary tissue pro-inflammatory cytokines observed in this study. However, oral TAD pretreatment not only reversed the DOX-induced hematological dysfunction and brought the values to normal but also caused a profound increase in the percentage myelocyte (MXD) count, which indicated a corresponding increase in eosinophils, monocytes, and basophils counts, and suggested activation of a general immunological response to the DOX intoxication by TAD pretreatment.

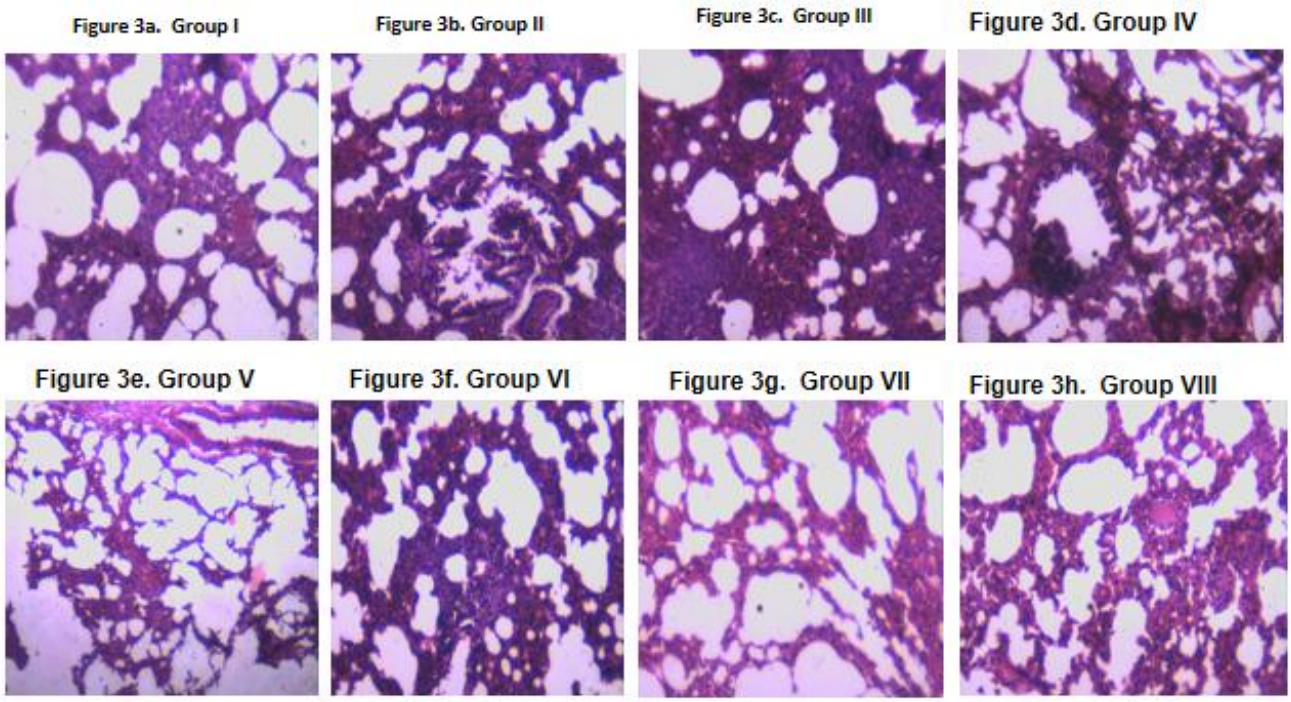

Figure 3.
A representative photographic section of (i). untreated normal control rat lung tissue showing normal bronchiole-alveolar architecture (x100 magnification, Hematoxylin & Eosin stains) **(3a)**; (ii). untreated DOX intoxicated rat lung tissue showing diffuse interstitial congestion with cellular infiltration; diffuse inter-alveolar septa thickening; and diffuse alveolar collapse (x100 magnification, Hematoxylin & Eosin stains) **(3b)**; (iii). 20 mg/kg/day SIL-only pretreated lung tissue showing normal pneumocytes, pulmonary interstitium, and inter-alveolar septa (x100 magnification, Hematoxylin & Eosin stains) **(3c)**; (iv). 20 mg/kg/day SIL pretreated + DOX-treated rat lung tissue showing moderate pulmonary interstitial congestion and focal bronchiolar congestion with cellular infiltration (x100 magnification, Hematoxylin & Eosin stains) **(3d)**; (v). 5 mg/kg/day TAD-only pretreated rat lung tissue showing normal bronchiolo-alveolar architecture and pulmonary interstitium (x100 magnification, Hematoxylin & Eosin stain) **(3e)**; (vi). 2.5 mg/kg/day TAD + 2.5 mg/kg DOX-treated rat lung tissue showing mild-to-moderate pulmonary interstitial congestion and focal alveolar collapse (x100 magnification, Hematoxylin & Eosin stain) **(3f)**; (vii). 5 mg/kg/day TAD + 2.5 mg/kg DOX-treated lung tissue showing mild pulmonary interstitial congestion with alveolar widening (x100 magnification, Hematoxylin & Eosin stains) **(3g)**; (viii). 10 mg/kg/day TAD + 2.5 mg/kg DOX-treated lung tissue showing mild pulmonary interstitial congestion and normal alveoli distribution (x100 magnification, Hematoxylin & Eosin stains) **(3h)**.

The blood HCO3- (reflective of arterial PCO2) and pH are often considered the most informative laboratory indicators of overall pulmonary function, especially in patients with pulmonary edema (Brinkman and Sharma, 2024). The primary disturbance of elevated arterial PCO2 is a decline in the ratio of arterial bicarbonate to arterial PCO2 with attendant decreased pH (Hirai *et al.*, 2019; Patel and Sharma, 2024). Thus, an arterial blood gas (ABG) and serum bicarbonate level are necessary to evaluate patients with suspected respiratory acidosis, a metabolic state marked by an elevated PCO_2, elevated HCO_3^-, and decreased pH (≤7.4) (Patel and Sharma, 2024). The fact that DOX-intoxication was associated with elevated serum HCO_3^- and reduced serum pH (7.4) suggested the presence of respiratory acidosis in the treated rats. The blood pH is known to have an inverse relationship with serum-ionized Ca^{2+} (Hamroun *et al.*, 2020). A rise in serum pH is known to promote the formation of calcium-albumin complexes and cause a decrease in ionized calcium levels; thus, a pH decrease may cause an increase in the free fraction of blood calcium. Therefore, an increase in ionized calcium levels (Hamroun *et al.*, 2020). In this study, repeated DOX treatment was associated with increased serum HCO_3^- and ionized Ca^{2+} and reduced serum pH, indicating the possible establishment of respiratory acidosis. Again, the fact that there were reversals in these measured pulmonary function parameters by TAD pretreatment suggests a potential protective effect of TAD against DOX-induced pulmonary dysfunction.

Another notable finding of this study is the effect of DOX toxicity on pulmonary tissue antioxidant activities. DOX intoxication was associated with significant reductions in the antioxidant enzyme (SOD, CAT, GPx, and GST) activities and significant increases and decreases in the pulmonary tissue MAD and GSH levels, respectively which are similar to those of other studies (Kuzu *et al.*, 2018; Kizir *et al.*, 2023). However, oral TAD pretreatment profoundly reverses the DOX-induced changes in the antioxidant profile.

The histopathological findings showed DOX-induced histological lesions such as diffuse interstitial congestion with cellular infiltration, diffuse inter-alveolar septa thickening and diffuse alveolar collapse. These observed histopathological changes remarkably improved with TAD pretreatment, highlighting the protective effect of TAD pretreatment in DOX-induced pulmonary toxicity.

Cancer chemotherapeutic agent acts on many proliferating cells or tissues, including the bone marrow. The bone marrow cells are responsible for red blood production, an important blood component. A complete

blood count assay is one of the most effective ways of monitoring drug toxicity (Sahota *et al.*, 2016; Erhabor *et al.*, 2020). Literature search shows that one of the DOX-induced hematotoxicities is bone marrow suppression that could manifest as anemia, thrombocytopenia, leukopenia, neutropenia, etc. (Isirima and Okoroafor, 2016; Ngatali *et al.*, 2022; Perpenia *et al.*, 2022). In this study, DOX intoxication did not cause leukopenia, thrombocytopenia, lymphocytopenia, myelocytosis, and neutrophila that could predispose the intoxicated rats to infections and spontaneous bleeding, respectively, from bone marrow suppression. However, oral TAD pretreatment reversed the DOX-induced hematological changes, restoring their values to about normal ranges, indicating the protective potential of graded oral TAD in preventing DOX-induced hematotoxicity.

In conclusion, this study highlights the possible chemopreventive potential of graded oral TAD against DOX-induced pulmonary and hematological toxicities, possibly mediated via anti-inflammatory and antioxidant mechanisms.

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Author contributions

AAA designed the experimental protocol, supervised the research, analyzed data and wrote the manuscript; FMO is an undergraduate student in AAA's Laboratory and carried out the experiment under AAA's supervision; OEO also contributed to the design of the experimental protocol and was involved in the manuscript editing; AOO read the slides, took the photomicrographs of the lung sections and was also involved in data analysis; IIO prepared the lung tissue slides for histopathological studies

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