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Tadalafil pretreatment attenuates doxorubicin-induced hepatorenal toxicity by modulating oxidative stress and inflammation in Wistar rats

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ABSTRACT

Doxorubicin (DOX) is a widely used anticancer agent, but its clinical application is limited by significant off-target hepatorenal toxicity. Tadalafil (TAD), a selective phosphodiesterase-5 inhibitor used mainly for erectile dysfunction and pulmonary arterial hypertension, has shown potential in reducing oxidative stress. This study investigated TAD's chemoprotective effects and underlying mechanisms in DOX-induced hepatorenal toxicity in rats over 12 days. Eight groups of six rats each were orally pretreated with sterile water, silymarin (SIL), or TAD one hour before receiving intraperitoneal injections of 2.5 mg/kg DOX. On the 13th day, the rats were humanely sacrificed under inhaled halothane anesthesia, and serum was collected for hepatic and renal function tests, while liver and kidney tissues were analyzed for antioxidant enzyme activity, pro-inflammatory cytokines assay, and histopathological evaluation. DOX successfully induced hepatorenal toxicity, evidenced by significant increases ($p<0.001$, $p<0.0001$) in serum K^+, urea, and creatinine levels, along with decreases in HCO_3^-, TCa^{2+}, and Cl^-. Tissue analysis showed reduced SOD, CAT, GST, and GPx activities, with elevated MDA and GSH levels. TAD pretreatment significantly ameliorated these biochemical alterations ($p<0.05$, $p<0.001$, $p<0.0001$), suggesting its potential as an effective chemoprophylactic adjuvant in the development of DOX-induced hepatorenal toxicity.

1. Introduction

Since its discovery in the 1960s and approval by the Food and Drug Administration in 1974 to treat cancer cases, doxorubicin (DOX), an anthracycline antibiotic cytotoxic, has been widely used in the clinical management of solid and hematological tumors, including soft tissues and bone sarcomas, cancers of the breast, ovary, bladder and the thyroid, lymphoblastic leukemia, acute myeloblastic leukemia, Hodgkin lymphoma, and small cell lung cancer [1,2].

DOX binds to topoisomerase II (an enzyme that relaxes supercoil in DNA transcription) and intercalates with deoxyribonucleic acid (DNA), thereby inhibiting DNA replication [3–5]. By intercalating the DNA, doxorubicin also induces histone expulsion from the transcriptionally active chromatin [6], this leads to the deregulation of DNA damage

Abbreviations: % Δbwt., percentage change in body weight; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bwt., average body weight; CAT, catalase; CGMP, cyclic guanosine monophosphate; Cl^-, chloride ion; DNA, Deoxyribose Nucleic Acid; DOX, Doxorubicin; ELISA, Enzyme-Linked Immunosorbent Assay; GGT, gamma-glutamyl transferase; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione S-transferase; HCO_3^-, bicarbonate ion; IL-1β, interleukin-1beta; IL-6, interleukin-6; INR, international normalized ratio; *i.p.*, intraperitoneal; K^+, potassium ion; LASUCOM, Lagos State University College of Medicine; LASU REC, Lagos State University Research Ethics Committee; MDA, malondialdehyde; Na^+, sodium ion; PDE5, phosphodiesterase-5; *p.o.*, *per os*; PT, prothrombin time; ROS, reactive oxygen species; S.D., standard deviation of the mean; S.E.M., standard error of the mean; SIL, Silymarin; SOD, superoxide dismutase; TAD, Tadalafil; TB, total bilirubin; TBA, thiobarbituric acid; TCA, 1,2-dichloro-4-nitrobenzene, trichloroacetic acid; TCa^{2+}, total calcium ion; TNF-α, tumor necrosis factor-alpha; TP, total protein.

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response, epigenome and transcriptome in DOX-exposed cells [7]. Also, DOX is known to increase the generation and release of the quinone-based free radicals that contribute to the drug's cytotoxic effects [8]. Despite its high efficacy, DOX use is limited by its off-target multi-organ, multi-directional cytotoxic effects and non-specificity to cancerous cells [9].

Tadalafi (TAD) (like its other congeners such as sildenafil and vardenafil) is a potent, selective, long-acting, and reversible oral inhibitor of phosphodiesterase-5 (PDE5) (the enzyme responsible for the metabolic degradation of cGMP) that has been approved primarily for the clinical management of erectile dysfunction [10–15] and pulmonary arterial hypertension [16–18]. Other studies have reported the therapeutic potentials of TAD in cisplatin-mediated nephrotoxicity [19] and testicular toxicity [20], adriamycin-induced nephrotic syndrome [21], DOX-induced cardiotoxicity [22] and heart failure [23], benign prostatic hypertrophy [24–26], and peripheral neuropathy and pain syndrome [27–29] and most recently type 2 diabetes mellitus [30,31]. Despite the increasingly wide application of TAD in disease management, its preventive potential against DOX-induced hepatorenal toxicity remains un-investigated. Therefore, this formed the basis of the current study that evaluates the therapeutic potential of TAD in mitigating the development of DOX-induced hepatorenal toxicities in rats using biochemical endpoints (hepatic and renal function tests, oxidative stress markers, pro-inflammatory markers) and histopathological endpoints.

2. Materials and methods

2.1. Experimental animals

Adult male Wistar Albino rats (aged 10–12 weeks old and body weight: 180–200 g) were from Bayo Farms, Sango-Ota, Ogun State, after obtaining ethical approval (with the reference number: LASU/23/REC/083) from the Lagos State University Research Ethics Committee (LASU REC), Lagos State University, Ojo, Lagos State, Nigeria. The choice of male rats was made based on the fact that preclinical evidences in rodents have shown that male rodents are more susceptible to DOX toxicities than the female rats due to the protection offered by the sex hormone, estrogen, which is predominant in the latter sex [32–35]. The rats were allowed to acclimatize for two (2) weeks in the Lagos State University College of Medicine (LASUCOM) Animal House before being used for experimentation. 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 (United States) Committee for the Update of the Guide for the Care and Use of Laboratory Animals [36]. The rats were maintained under standard laboratory conditions, with rats chow and potable drinking water freely made available throughout the study.

2.2. Body weight measurement

At both the commencement and end of the experiment, rat body weights were taken using a digital rodent weighing scale (Virgo Electronic Compact Scale®, New Delhi, India). The values obtained were expressed in grams (g).

2.3. Drug pretreatment and experimental induction of doxorubicin-induced hepatorenal toxicity in rats

DOX-induced hepatorenal toxicity induction was done using the method earlier described by Adeneye *et al.* [37]. 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 body weight of the sample population.

The treatment of each group based on their treatment drugs is shown in Table 1. The experimental rats were orally treated with sterile water, SIL (Silybon-140®, Micro Labs Limited, 92 Sipcot Hosur-635126, India), and TAD (Honnonil®, Lyn-Edge Pharmaceutical Limited, Chevron Alternative Route, Poroku, Lekki, Lagos State, Nigeria) 1 hour before the intraperitoneal injection of 2.5 mg/kg of DOX (Oncodox-50®, Cipla Limited, Plot No. 5, S-103 Verna, Goa403–722, India). The choices of doses of drugs and duration treatment were based on our previous studies and preliminary studies earlier done [19,37].

Table 1
Groups and their drug treatments.

Groups	Drug Treatments
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
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
III	20 mg/kg/day SIL in sterile water given *p.o.* daily + 1 ml/kg/day sterile water *i.p.* on alternate days for 12 days
IV	20 mg/kg/day SIL 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
V	5 mg/kg/day TAD in sterile water given *p.o.* daily + 1 ml/kg/day sterile water given *i.p.* on alternate days for 12 days
VI	2.5 mg/kg/day TAD 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
VII	5 mg/kg/day TAD 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
VIII	10 mg/kg/day TAD in sterile water given *p.o.* + 2.5 mg/kg/day *DOX* in sterile water given *i.p.* on alternate days for 12 days

2.4. 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 controlled and light inhaled halothane anesthesia for whole blood sample collection directly from the heart with fine 21 G injectable 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).

Following the humane sacrifice, the carcasses were evacuated and duly processed by the trained and certified Animal House Attendants in the collection, storage, safe disposal, and treatment of medical and toxic waste, including the safe disposal of dead experimental animals [19,37].

2.5. Calculation of percentage weight changes (%Δbwt.)

The percentage weight change (%Δbwt.) was calculated as a ratio of the difference between the final and initial body weights and the initial body weight multiplied by 100 as previously described by Adeneye *et al.* [19,37] which is expressed mathematically in the equation:

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

2.6. 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 as previously described by Adeneye *et al.* [19,37]. This is expressed mathematically as:

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

2.7. Blood sample collection

For each rat, 4–5 ml of whole blood was collected directly from the heart chamber using a 21 G needle mounted on a 5 ml syringe into a

plain sample (for kidney and renal function assays). The collected blood samples were allowed to clot at 4 °C for 4 hours before being centrifuged at 5000 revolutions/min for 5 minutes to separate the sera from the other clotted blood components. Thereafter, the serum was separated into another plain sample bottle for the hepatic and renal function assay as previously described by Adeneye *et al.* [37].

2.8. Determination of renal function tests

The obtained sera were used for the biochemical analyses of the liver function test {liver enzymes – [alanine aminotransferase (ALT), aspartate aminotransferase (AST); alkaline phosphatase (ALP)], total protein (TP), albumin (ALB), and total bilirubin (TB)} and renal function test {electrolytes – [sodium (Na^+), potassium (K^+), bicarbonate (HCO_3^-)]; urea and creatinine}. The assays were conducted using standard bioassay procedures and the Manufacturer's instructions on the enclosed leaflets in the commercial diagnostic test kits (Randox Diagnostics Test Kits®, Randox Laboratories Limited, Crumlin, United Kingdom). The serum creatinine and urea measurements were based on the principle of Jaffe's reaction while liver aminotransferases measurement were based on Reitman and Frankel's colorometric methods described by Rosner and Bolton (2005) [38].

2.9. Liver and kidney tissue antioxidant enzymatic assays and pro-inflammatory markers

The activities of liver and the kidneys tissue oxidative stress marker enzymes (GSH, MDA, CAT, SOD, GST, and GPx) were determined using the procedures earlier described by Olorundare *et al.* [39,40]. The unit of activity of the antioxidant enzyme activities was 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 liver and kidney tissues were also determined using rat-specific commercial ELISA kits sourced from ElabScience (14780 Memorial Drive, Suite 105, Houston, Texas, 77079, USA) based on the principle enzyme-linked immunosorbent assay as described by Haapakoski *et al.* [41].

2.10. Histopathological studies of the liver and kidney tissues

The remaining one-half of the kidneys and liver were dissected out and preserved in a 10 % formo-saline solution. The slide preparation of the tissues and the reading of the slides were done using the procedures described by Olorundare *et al.* [39,40].

2.11. Data analysis

The data for the average 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 $p<0.0001$.

3. Results

3.1. Effects of tadalafil pretreatment and doxorubicin intoxication on the average body weight and weight changes in treated rats

Table 2 shows that alternate-day intraperitoneal DOX injections 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 ± 20.68 g, 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
Effects of doxorubicin intoxication, oral silymarin and graded tadalafil pre-treatments on the average body weights (bwt.), percentage body weight changes (% Δbwt.) and relative liver and kidney weights in treated rats.

Treatment Groups	initial bwt. (g)	final bwt. (g)	% Δbwt	relative liver weight	relative kidney weight
I	173.0 ±21.2	182.5 ±22.6	05.1 ±03.9	03.0±0.7	00.7±0.1
II	192.2 ±16.6	137.4 ±20.7^{c-}	28.2 ±04.2^{c-}	03.7 ± 0.1^{b+}	00.7 ± 0.1
III	193.7 ±05.9	207.2 ±12.1$^{a+,a\#}$	07.10 ±06.1$^{c\#}$	03.1 ± 0.4^{b*}	00.6 ± 0.1
IV	186.8 ±05.6	143.1 ±25.7^{c-}	−16.4 ±22.0^{c-}	03.2 ± 0.5^{a*}	00.7 ± 0.0
V	200.7 ±22.8	218.8 ±18.6$^{a+,c\#}$	05.8 ±04.4$^{c\#}$	02.6±0.1^{c*}	00.6±0.1
VI	204.7 ±21.6	158.5 ±24.8^{c-}	−22.8 ±06.8^{c-}	03.3±0.4^{a*}	00.7±0.1
VII	195.5 ±19.5	154.0 ±26.1^{b-}	−21.6 ±06.6^{c-}	03.5±0.3^{a*}	00.7±0.1
VIII	199.7 ±20.2	150.1 ±17.9^{c-}	−25.0 ±03.2^{c-}	03.0 ± 0.6^{b*}	00.6 ± 0.2

$^{b-}$ 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.
$^{b+}$ and $^{a-}$ represent significant increase and decrease at $p<0.001$ and $p<0.05$, respectively, when compared to untreated normal control (Group I) values while ^{a*} and ^{b*} represent significant decreases at $p<0.05$ and $p<0.001$, respectively, when compared to the untreated DOX intoxicated (Group II) values.
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 SIL 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 SIL 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 TAD 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 TAD 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 TAD 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 TAD in sterile water given *p.o.* + 2.5 mg/kg/day DOX in sterile water given *i.p.* on alternate days for 12 days.

Daily oral pre-treatments with SIL (used as standard 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 pre-treated, DOX intoxicated rats (Table 2).

3.2. Tadalafil pretreatment and doxorubicin intoxication on the relative liver and kidney weight in treated rats

On the relative liver weight, DOX intoxication caused a significant increase (at $p<0.001$) in the relative liver weights (03.7 ± 0.1) (Table 2). However, pre-treatment with oral SIL and oral doses 2.5 mg/kg body weight/day and 5 mg/kg body weight/day of TAD significantly

($p<0.05$) attenuated the DOX-induced increase in the relative liver weight value (3.2 ± 0.5, 3.3 ± 0.4, and 3.5 ± 0.3, respectively) while the highest TAD dose (10 mg/kg body weight/day) significantly ($p<0.05$) further reduced the relative weight value (3.0 ± 0.6) (Table 2).

Conversely, DOX intoxication did not significantly ($p>0.05$) alter the relative organ weight for the kidneys (0.7 ± 0.1) (Table 2). Similarly, oral daily pre-treatment with SIL and graded TAD did not significantly ($p>0.05$) alter the relative kidney weights (0.7 ± 0.0; 0.7 ± 0.1; 0.7 ± 0.1; and 0.6 ± 0.2, respectively) (Table 2).

3.3. Effects of doxorubicin intoxication and tadalafil pretreatment on the liver function parameters (serum liver enzymes, total protein, albumin and total bilirubin) in treated rats

Repeated alternate-day treatment with intraperitoneal injections of 2.5 mg/kg body weight DOX resulted in significant ($p<0.0001$) increases in the liver enzymes {aspartase aminotransferase (AST) and alanine aminotransferase (ALT)}, and total bilirubin (TB) levels while causing non-significant ($p>0.05$) alterations in the serum alkaline phosphatase (ALP) levels when compared to untreated normal (Group I) values (Table 3). DOX intoxication also caused significant ($p<0.001$, $p<0.0001$) decreases in the serum total proteins and albumin when compared to untreated control (Group I) values (Table 3).

Oral SIL and graded oral TAD pre-treatments resulted in significant decreases ($p<0.001$ and $p<0.0001$) in the serum ALT, ALP, total protein (TP) and TB levels while causing profound increases ($p<0.0001$) in the serum albumin (ALB) levels in DOX intoxicated rats when compared with the values for untreated positive control (Group II – untreated DOX intoxicated) rats (Table 3).

3.4. Tadalafil pretreatment and doxorubicin intoxication on the renal function parameters (serum electrolytes, urea and creatinine) in treated rats

Repeated alternate-day treatment with intraperitoneal injections of 2.5 mg/kg body weight DOX for 12 successive days resulted in significant ($p<0.001$) increases in the serum potassium (K^+), urea, and creatinine while causing significant ($p<0.05$, $p<0.001$, $p<0.0001$) decreases in the serum total calcium (TCa^{2+}), bicarbonate (HCO_3^-), and chloride (Cl^-) levels, respectively, when compared to the untreated normal control (Group I) values (Table 4). With oral SIL and graded TAD pretreatments, there were significant decreases ($p<0.05$, $p<0.001$, and $p<0.0001$) in the serum K^+, urea, and creatinine when compared to the untreated positive control (Group II) values (Table 4). However, neither DOX intoxication nor oral pretreatments with SIL or graded oral TAD caused significant ($p>0.05$) alterations in the serum sodium (Na^+) levels (Table 4).

3.5. Effects of tadalafil pretreatment and doxorubicin intoxication on the hepatic tissue superoxidase dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione-S-transferase (GST), glutathione peroxidase (GPx) activities and reduced glutathione (GSH) levels in treated rats

Table 5 represents the effect of DOX intoxication and oral pretreatments with SIL and graded doses of TAD on the hepatic antioxidant activities. As shown in Table 5, repeated alternate-day treatment with intraperitoneal injections of 2.5 mg/kg body weight DOX for 12 days resulted in significant ($p<0.0001$) decreases in the hepatic SOD, CAT, GST, and GPx activities and significant ($p<0.05$) decrease in the hepatic GSH levels. Conversely, DOX intoxication was associated with a significant ($p<0.0001$) increase in the hepatic tissue MDA activities (Table 5). However, oral SIL and graded TAD pretreatments resulted in significant increases ($p<0.001$ and $p<0.0001$) in the hepatic tissue SOD, CAT, GST, and GPx activities, restoring their activities to normal (Table 6). Similarly, oral SIL, and graded TAD pretreatments also significantly ($p<0.05$, $p<0.001$, and $p<0.0001$) increased the GSH levels (Table 5). Conversely, oral SIL, and graded TAD pretreatments also significantly ($p<0.0001$) decreased the MDA activities in the treated hepatic tissues, with 10 mg/kg body weight/day TAD eliciting the most effective restoration activities when compared with the untreated DOX-intoxicated control (Group II) values (Table 5).

3.6. Effects of doxorubicin intoxication and tadalafil pretreatment on the renal tissue superoxidase dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathinone-s-transferase (GST), glutathione peroxidase (GPx) activities and reduced glutathione (GSH) levels in treated rats

Table 6 represents the effect of DOX intoxication and oral SIL and graded TAD pretreatments on the renal tissue antioxidant profile. As shown in Table 6, repeated alternate-day treatment with intraperitoneal injections of 2.5 mg/kg body weight DOX for 12 days resulted in

Table 3
Graded oral tadalafil and silymarin pretreatments and DOX intoxication on the liver function parameters (liver enzymes, total proteins, albumin and total bilirubin) in treated rats.

Treatment Group	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/L)	ALB (g/L)	TB (μmol/L)
I	59.0±12.0	56.8±11.2	147.8±8.4	73.0±2.5	36.1±2.9	2.0±0.7
II	152.2±3.0c+	187.9±2.6c+	151.0±6.0	56.0±0.3b-	26.5±0.5c-	13.0±0.2c+
III	38.2±4.9c*	42.4±7.5c*	185.5±17.4c+	69.7±2.8	37.9±0.2c#	0.7±0.2c*
IV	236.3±15.5c+	99.2±12.2c#	78.1±3.7c*	58.1±3.7b-	29.1±1.2c-	5.7±2.5c*
V	170.6±20.9c+	67.0±3.6c*	117.2±6.8a*	70.9±2.3b*	38.9±1.9c#	1.2±0.4c*
VI	339.6±41.1c*	84.1±15.3b*	53.5±12.4c*	43.5±9.1c*	20.8±4.3c*	2.2±0.8c*
VII	267.4±11.3c	74.2±9.8c*	43.8±9.8c*	54.3±6.2c*	27.6±3.2	2.9±1.6c*
VIII	229.5±13.3c#	57.7±13.8c*	45.2±6.2c*	53.0±2.5c*	38.2±4.9c*	2.7±1.0c*

$^{c+}$ represents a significant increase at $p<0.0001$ while $^{b-}$ and $^{c-}$ represent significant decreases at $p<0.001$ and $p<0.0001$, respectively, when compared to untreated normal (Group I) rats. ^{b*} and ^{c*} represents significant decreases at $p<0.001$ and $p<0.0001$, respectively, while $^{c\#}$ represents a significant increase at $p<0.001$ when compared to untreated DOX intoxicated control (Group II) values.
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 SIL 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 SIL 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 TAD 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 TAD 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 TAD 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 TAD in sterile water given *p.o.* + 2.5 mg/kg/day DOX in sterile water given *i.p.* on alternate days for 12 days.

Table 4
Graded oral tadalafil and silymarin pretreatments and doxorubicin intoxication on the renal function parameters {electrolytes (Na^+, K^+, Cl^-, HCO_3^-, and TCa^{2+}), urea and creatinine} in treated rats.

Treatment Groups	Na+ (mmol/L)	K+ (mmol/L)	Cl- (mmol/L)	HCO3-(mmol/L)	TCa2+ (mmol/L)	Urea (mmol/L)	Creatinine (μmol/L)
I	143.3±0.5	10.7±0.5	101.6±0.3	23.7±0.9	2.3±0.1	5.5±0.5	30.6±2.7
II	136.1±4.1	17.7±3.3b+	94.9±2.2c-	18.4±2.4b-	2.1±0.0a-	17.7±0.2c+	54.5±0.6c+
III	143.4±0.4	8.9±0.7b*	99.4±0.4	25.0±0.6b#	2.3±0.1	6.1±0.5c*	22.1±2.6c*
IV	142.2±0.7	9.3±0.3b*	104.2±0.9c#	23.5±1.5b#	2.4±0.1a#	11.0±2.3b*	39.8±5.3b*
V	143.6±0.5	7.3±0.2b*	100.2±0.6a*	28.1±0.8b#	2.4±0.0a#	6.4±0.7c*	30.6±2.7b*
VI	141.1±4.4	12.8±1.4	106.5±0.7c#	20.7±1.9	2.2±0.3	12.3±1.4b*	34.8±2.5b*
VII	140.3±0.8	10.9±9.8a*	103.4±1.0c#	23.0±1.7b#	2.4±0.1a#	11.0±2.1b*	34.5±2.3b*
VIII	143.0±2.1	9.7±0.4b*	107.5±2.1c#	25.6±1.0b#	2.4±0.1a#	10.7±1.2b*	28.5±1.9c*

$^{b+}$ and $^{c+}$ represent significant increases at $p<0.001$ and $p<0.0001$, respectively, while $^{b-}$ and $^{c-}$ represent significant decreases at $p<0.001$ and $p<0.0001$, respectively, when compared to untreated normal (Group I) rats. ^{b*} and ^{c*} represents significant decreases at $p<0.001$ and $p<0.0001$, respectively, 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.
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 SIL 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 SIL 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 TAD 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 TAD 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 TAD 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 TAD in sterile water given *p.o.* + 2.5 mg/kg/day DOX in sterile water given *i.p.* on alternate days for 12 days.

Table 5
Effects of doxorubicin intoxication, oral silymarin and graded oral tadalafil pretreatments on the hepatic tissue antioxidant profile in treated rats.

Treatment Groups	SOD (U/L)	CAT (U/L)	MDA (U/L)	GST (g/L)	GPx (g/L)	GSH (μmol/L)
I	2.9 ±0.2	11.2 ±0.1	2.0 ±0.0	34.1 ±1.2	71.6 ±1.1	53.9 ±0.8
II	2.1 ±0.0^{c-}	7.0 ±0.2^{c-}	4.7 ±0.0^{c+}	26.1 ±1.3^{b-}	54.2 ±2.1^{c-}	42.1 ±0.1^{a-}
III	3.2 ±0.1$^{c\#}$	11.6 ±0.6$^{c\#}$	1.7 ±0.0^{c*}	39.8 ±1.1$^{c\#}$	80.5 ±0.4$^{c\#}$	59.7 ±0.3$^{c\#}$
IV	3.5 ±0.1$^{c\#}$	11.3 ±0.6$^{c\#}$	1.7 ±0.0^{c*}	47.0 ±1.2$^{c\#}$	88.1 ±0.3$^{c\#}$	63.9 ±0.2$^{c\#}$
V	3.7 ±0.2$^{c\#}$	9.3 ±0.3$^{a\#}$	1.8 ±0.0^{c*}	45.7 ±1.6$^{c\#}$	89.2 ±0.5$^{c\#}$	65.7 ±0.2$^{b\#}$
VI	4.6 ±0.3$^{c\#}$	9.4 ±0.13$^{a\#}$	2.2 ±0.1^{b*}	57.1 ±3.7$^{c\#}$	94.9 ±0.3$^{c\#}$	72.1 ±0.2$^{c\#}$
VII	4.5 ±0.3$^{c\#}$	8.8 ±0.2$^{a\#}$	2.2 ±0.0^{b*}	57.9 ±1.7$^{c\#}$	98.6 ±0.5$^{c\#}$	74.0 ±0.4$^{c\#}$
VIII	4.6 ±0.2$^{c\#}$	8.8 ±0.2$^{a\#}$	2.2 ±0.0^{b*}	57.9 ±1.7$^{c\#}$	98.6 ±0.5$^{c\#}$	74.0 ±0.4$^{c\#}$

$^{c+}$ represents a significant increase at $p<0.0001$ while $^{a-, b-}$ and $^{c-}$ represent significant decreases at $p<0.05$, $p<0.001$ 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 $^{a\#, b\#}$ 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.
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 SIL 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 SIL 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 TAD 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 TAD 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 TAD 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 TAD in sterile water given *p.o.* + 2.5 mg/kg/day DOX in sterile water given *i.p.* on alternate days for 12 days.

Table 6
Graded oral tadalafil and silymarin pretreatments and doxorubicin intoxication on the renal tissue antioxidant profile in treated rats.

Treatment Groups	SOD (U/L)	CAT (U/L)	MDA (U/L)	GST (g/L)	GPx (g/L)	GSH (μmol/L)
I	3.7 ±0.2	15.1 ±0.1	1.9 ±0.0	40.0 ±2.5	62.7 ±2.0	47.2 ±1.5
II	2.5 ±0.1c-	9.8 ±0.3c-	3.0 ±0.0c+	23.0 ±0.9c-	44.4 ±0.5c-	38.3 ±0.1a-
III	3.6 ±0.2c#	15.3 ±0.4c#	2.1 ±0.0b*	38.0 ±1.2b#	68.3 ±0.9c#	50.4 ±0.8c#
IV	3.7 ±0.2c#	14.9 ±0.3c#	2.3 ±0.0b*	48.2 ±2.8c#	71.2 ±1.3c#	52.0 ±1.0c#
V	3.5 ±0.2c#	14.8 ±0.4c#	2.3 ±0.0b*	38.3 ±1.9b#	70.9 ±0.7c#	52.5 ±0.5c#
VI	4.4 ±0.2c#	14.8 ±0.2c#	2.5 ±0.0a*	46.5 ±1.9c#	71.2 ±1.1c#	53.9 ±0.5c#
VII	4.4 ±0.1c#	14.0 ±0.3c#	2.4 ±0.0b*	47.9 ±1.7c#	71.6 ±0.7c#	54.5 ±0.7c#
VIII	3.9 ±0.2c#	14.3 ±0.3c#	2.2 ±0.1c*	44.0 ±2.3c#	71.4 ±0.5c#	53.1 ±0.4c#

$^{c+}$ represents a significant increase at $p<0.0001$ while $^{a-}$ and $^{c-}$ represent significant decreases at $p<0.05$, $p<0.05$ and $p<0.0001$, respectively, when compared to untreated normal (Group I) rats. ^{a*}, ^{b*} and ^{c*} represent significant decreases at $p<0.05$, $p<0.001$ 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.
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 SIL 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 SIL 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 TAD 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 TAD 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 TAD 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 TAD in sterile water given *p.o.* + 2.5 mg/kg/day DOX in sterile water given *i.p.* on alternate days for 12 days.

significant ($p<0.0001$) decreases in the renal SOD, CAT, GST, and GPx activities and significant ($p<0.0001$) decrease in the renal GSH levels. Conversely, DOX intoxication was associated with a significant increase ($p<0.0001$) in the renal tissue MDA activities when compared to untreated normal control (Group 1) values (Table 6). However, oral SIL and graded TAD pretreatments resulted in significant increases ($p<0.001$ and $p<0.0001$) in the renal tissue SOD, CAT, GST, and GPx activities, restoring their activities to normal values (Table 6). Similarly, oral pretreatments with SIL, and TAD fixed dose combination also significantly ($p<0.0001$) increased the renal tissue GSH levels when compared to the untreated DOX-intoxicated (Group II) values (Table 6). Conversely, oral SIL, and graded TAD also significantly ($p<0.05$, $p<0.001$, $p<0.0001$) decreased the MDA activities in the DOX-intoxicated renal tissues, with 10 mg/kg body weight/day and fixed-dose combination eliciting the most effective restoration activities, when compared with the untreated DOX-intoxicated control (Group II) values (Table 6).

3.7. Effects of tadalafil and silymarin pretreatment and doxorubicin intoxication serum pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) on the hepatic and renal tissues of treated rats

In the untreated DOX intoxicated group, there were significant ($p<0.001$ and $p<0.0001$) increases in the hepatorenal levels of the measured pro-inflammatory cytokines: IL-1β, IL-6, and TNF-α levels were recorded compared to the untreated normal rats (Tables 7 and 8). However, oral SIL and graded TAD pretreatments caused significant ($p<0.05$, $p<0.001$, and $p<0.0001$) decrease in hepatorenal concentrations of IL-1β, IL-6, and TNF-α and reverting the values to normal compared to untreated DOX intoxicated group (Tables 7 and 8).

Table 7
Effects of doxorubicin intoxication, oral silymarin and graded oral tadalafil pretreatments on the hepatic pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in treated rats.

Treatment Groups	IL-1β(pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)
I	26.0 ± 2.5	16.1 ± 1.4	19.7 ± 0.9
II	74.4 ± 5.1c+	37.0 ± 3.0b+	70.1 ± 3.4c+
III	34.9 ± 3.2c*	19.0 ± 1.0b*	22.9 ± 2.3c*
IV	38.2 ± 4.3c*	21.4 ± 0.9a*	33.6 ± 2.3c*
V	26.9 ± 2.1c*	18.0 ± 0.6b*	19.8 ± 0.9c*
VI	41.1 ± 2.3b*	23.0 ± 0.8a*	35.2 ± 2.0c*
VII	34.4 ± 1.9c*	21.4 ± 1.2c*	29.3 ± 2.0b*
VIII	28.3 ± 2.6c*	19.6 ± 0.9b*	22.7 ± 0.8c*

$^{b+}$ and $^{c+}$ represent significant increases at $p<0.001$ and $p<0.0001$, respectively, when compared to 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 the untreated DOX intoxicated (Group II) values.
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 SIL 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 SIL 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 TAD 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 TAD 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 TAD 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 TAD in sterile water given *p.o.* + 2.5 mg/kg/day DOX in sterile water given *i.p.* on alternate days for 12 days.

Table 8
Effects of doxorubicin intoxication, oral silymarin and graded oral tadalafil pretreatments on the renal pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in treated rats.

Treatment Groups	IL-1β(pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)
I	22.30 ± 1.21	15.37 ± 0.78	20.12 ± 1.24
II	41.60 ± 1.88^{c}+	27.53 ± 0.98^{c}+	37.80 ± 1.39c+
III	28.62 ± 1.12^{c}*	19.27 ± 2.07^{c}*	22.28 ± 0.95^{c}*
IV	32.77 ± 1.24^{b}*	22.10 ± 0.92^{a}*	30.72 ± 0.91^{a}*
V	26.13 ± 1.61^{c}*	15.90 ± 0.97^{c}*	21.48 ± 0.38^{c}*
VI	34.95 ± 1.20^{b}*	26.82 ± 0.69	28.90 ± 1.68^{b}*
VII	28.85 ± 0.49^{c}*	22.56 ± 1.32^{a}*	28.17 ± 1.01^{b}*
VIII	25.65 ± 1.14^{c}*	19.37 ± 0.60^{b}*	22.68 ± 1.79^{c}*

$^{b+}$ and $^{a-}$ represent significant increase and decrease at $p<0.001$ and $p<0.05$, respectively, when compared to untreated normal control (Group I) values while ^{a}* and ^{b}* represent significant decreases at $p<0.05$ and $p<0.001$, respectively, when compared to the untreated DOX intoxicated (Group II) values.
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 SIL 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 SIL 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 TAD 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 TAD 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 TAD 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 TAD in sterile water given *p.o.* + 2.5 mg/kg/day DOX in sterile water given *i.p.* on alternate days for 12 days.

3.8. Effects of tadalafil and silymarin pretreatment and doxorubicin intoxication histopathogical studies on the hepatic and renal tissues of treated rats

Repeated DOX injection to the treated rat liver was associated with severe central hepatic vascular congestion (indicated in red thick arrow) and hepatocyte congestion and vacuolar degeneration (indicated in black thick arrow) (Fig. 1b) compared to the untreated normal liver that showed normal hepatic histoarchitecture (Fig. 1a). Oral SIL and TAD pretreatments in the DOX-intoxicated rats significantly improved the hepatic vascular congestion as well as the hepatocyte congestion and vacuolation (Figs. 1c-1h).

Similarly, repeated DOX injection caused diffuse glomerular atrophy and severe tubulointerstitial congestion in the treated rat kidney (Fig. 2b) compared with the untreated control kidneys and has normal renal histoarchitecture (Fig. 2a). Pretreatment with SIL in normal and DOX-intoxicated rat kidneys was associated with moderate tubulointerstitial congestion and focal glomerular thickening (Fig. 2d) when compared to SIL-only pretreated rat kidneys that showed normal glomeruli and convoluted tubules (Fig. 2c). However, oral pretreatment with 2.5 mg/kg/day, 5 mg/kg/day and 10 mg/kg/day TAD to DOX-intoxicated rats, there were remarkable improvements in the DOX-associated distortion in the renal histoarchitecture of the treated rats (Figs. 2f-2h) when compared to the 5 mg/kg/day TAD-only pretreated rat kidney which showed occasional glomerular atrophy (Fig. 2e).

4. Discussion

In this study, repeated intraperitoneal injections of 2.5 mg/kg of DOX following the oral SIL and TAD pretreatments for 12 days were associated with weight loss. This finding is similar to other studies that reported weight loss as a side-effect of DOX therapy [42,43] that was reportedly mediated through the induction of fatigue, anorexia, and skeletal muscle atrophy [43]. The recorded body weight loss was inverse

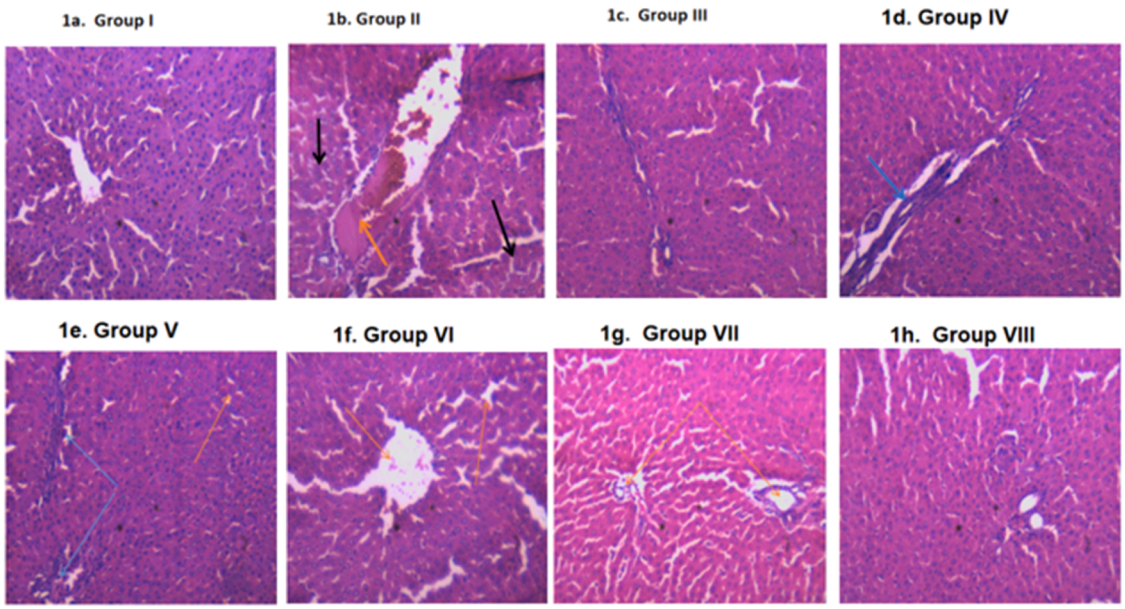

Fig. 1. A representative photographic section of (i). untreated normal control rat hepatic tissue showing normal hepatic architecture (x100 magnification, Hematoxylin & Eosin stains) (1a); (ii). untreated DOX intoxicated rat hepatic tissue showing severe central hepatic vascular congestion (indicated in red thick arrow) and hepatocyte congestion and vacuolation (indicated in black thick arrow) (x100 magnification, Hematoxylin & Eosin stains) (1b); (iii). 20 mg/kg/day SIL-only pretreated hepatic tissue showing normal hepatic vasculature and hepatocytes (x100 magnification, Hematoxylin & Eosin stains) (1c); (iv). 20 mg/kg/day SIL pretreated + DOX-treated rat hepatic tissue showing moderate lymphocytic infiltration of the hepatic portal triad (indicated in thick blue arrow) (x100 magnification, Hematoxylin & Eosin stains) (1d); (v). 5 mg/kg/day TAD-only pretreated rat hepatic tissue showing mild hepatic congestion (indicated in red thin arrow) (x100 magnification, Hematoxylin & Eosin stain) (1e); (vi). 2.5 mg/kg/day TAD + 2.5 mg/kg DOX-treated rat hepatic tissue showing moderate hepatic vascular congestion (indicated in red thin arrow) and mild lympohocytic infiltration of the hepatic vasculature (indicated in blue thin arrow) (x100 magnification, Hematoxylin & Eosin stain) (1 f); (vii). 5 mg/kg/day TAD + 2.5 mg/kg DOX-treated hepatic tissue showing mild portal triad congestion (indicated in red thin arrow) (x100 magnification, Hematoxylin & Eosin stains) (1 g); (viii). 10 mg/kg/day TAD + 2.5 mg/kg DOX-treated hepatic tissue showing relatively normal hepatocytes and hepatic vasculature (x100 magnification, Hematoxylin & Eosin stains) (1 h). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with a corresponding increment in the relative liver weight, which could have resulted from disproportional changes in the liver weight to body weight ratio. The fact that did not reverse the DOX-induced weight loss in DOX-treated rats indicated that TAD does not possess intrinsic properties to reverse DOX-induced weight loss. Similarly, the fact that DOX did not cause a significant reduction in the relative kidney weight suggested that DOX could have some sparing effects on the kidney tissue mass.

The liver remains an essential body organ that is involved in food and drug metabolism, detoxification, glucose homeostasis, blood clotting regulation, albumin production, bile synthesis, and vitamin and mineral storage [44]. Inherent in the liver tissues are liver enzymes that help regulate its functions including bodily chemical reactions such as drug metabolism. The integrity of the liver enzymes is measured and monitored through a group of liver enzyme tests known as the liver function test. The liver function tests typically include alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, prothrombin time (PT), the international normalized ratio (INR), total protein and albumin [45]. These tests can help determine an area of the liver where damage may be taking place and, depending on the pattern of elevation, can help organize a differential diagnosis [45]. Some of these tests are associated with functionality (e.g. albumin), some with cellular integrity (e.g., transaminase), while others are linked to the biliary tract (GGT and ALP) [46]. Often in liver injury or liver disease, these liver enzymes are elevated due to their release from the hepatic cells where they are generally stored in the blood circulation [47]. ALT and AST are found inside hepatocytes and are released when the liver is damaged while ALP another enzyme found in the cell lining of the biliary duct of the liver, is also elevated during hepatobiliary injury [47]. Cancer chemotherapeutic agents especially alkylating agents and notoriously DOX are generally reputed for altering the liver enzyme functions and elevating their serum levels [48]. In this study, DOX-induced hepatotoxicity was evidenced by an increment in serum ALT and AST activities as well as total bilirubin in DOX-treated rats compared to the negative control rats, indicating serious liver injury. These findings are in complete agreement with reports of other studies [49–55]. However, oral pretreatment with TAD reversed the hepatic damage by decreasing the serum ALT, and total bilirubin levels and increasing the serum total proteins and albumin levels in the same way as silymarin (a standard hepatoprotective antioxidant drug). These findings are indicative of the protective potential of TAD against DOX-induced hepatotoxicity. This result is similar to that earlier reported by El Khouly and Ebrahim [53] where TAD pretreatment for 7 days offered protection against γ-rays-induced hepatic injury in rats as well as the report of Mansour *et al.* [54] which reported TAD's anti-inflammatory and anti-fibrotic effects in thioacetamide-induced liver fibrosis in rats. However, it is notable from the findings of this study that neither SIL nor TAD pretreatments ameliorated the serum AST levels in the DOX intoxicated rats. This observation suggests that the elevated AST levels could be from extrahepatic sources. Literature shows that alanine aminotransferase (ALT) is a more specific and reliable marker of liver disease or toxicity than aspartase aminotransferase (AST) as the activity of the latter could be extrahepatic, being abundant also in extrahepatic tissues such as skeletal muscle, kidneys, brain, erythrocytes and lungs [55]. Thus, the significantly elevated serum AST levels could be from these extrahepatic sources. Similarly, according to a study conducted by Bektas *et al.* [56] to determine the effects of TAD and pentoxifylline on apoptosis and nitric oxide synthase in liver ischemia/reperfusion injury showed that there was an increase in the levels of ALT, AST, and ALP enzymes in both the 10 mg/kg and 2.5 mg/kg of TAD groups. These findings were further corroborated by the findings of Onyilo and Samuel (2024) [57]. Our

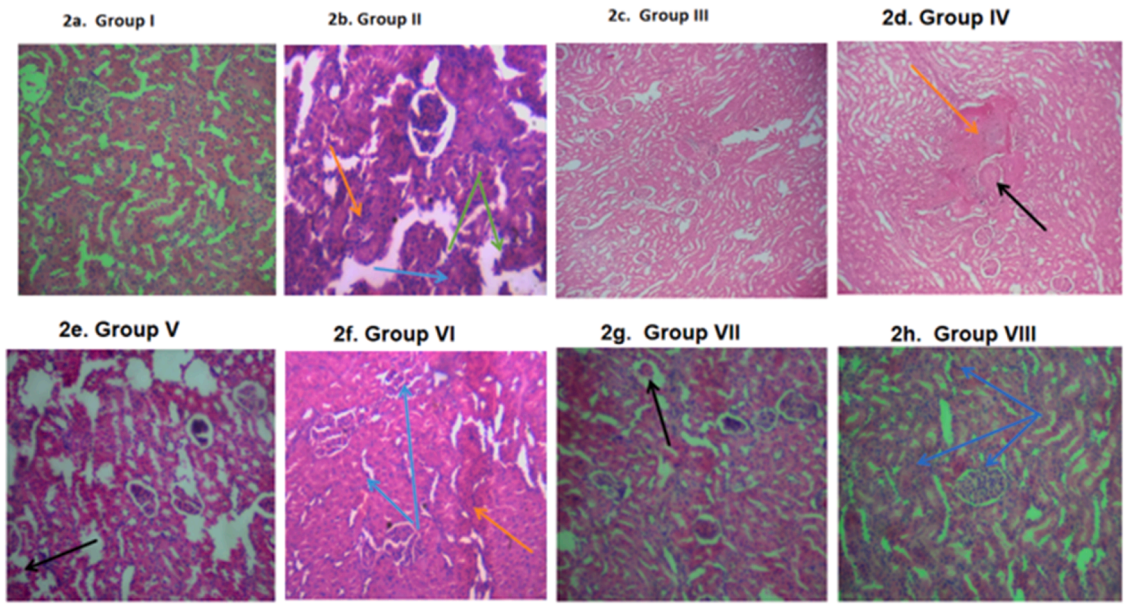

Fig. 2. A representative photographic section of (i). untreated normal control rat kidney tissue showing normal tubulo-glomerular architecture (x100 magnification, Hematoxylin & Eosin stains) (2a); (ii). untreated DOX intoxicated rat kidney tissue showing diffuse glomerular atrophy (indicated by green thick arrows) and severe tubulo-interstitial congestion (indicated by red thick arrows) with lymphocytic infiltration (indicated by blue thick arrows) (x100 magnification, Hematoxylin & Eosin stains) (2b); (iii). 20 mg/kg/day SIL-only pretreated renal tissue showing normal glomeruli and convoluted tubules (x100 magnification, Hematoxylin & Eosin stains) (2c); (iv). 20 mg/kg/day SIL pretreated + DOX-treated rat kidney tissue showing moderate tubulo-interstitial congestion (indicated by red thick arrow) and focal glomerular thickening (indicated by black thick arrow) (x100 magnification, Hematoxylin & Eosin stains) (2d); (v). 5 mg/kg/day TAD-only pretreated rat renal tissue showing focal glomerular atrophy (indicated by black thick arrow) and normal interstitium (x100 magnification, Hematoxylin & Eosin stain) (2e); (vi). 2.5 mg/kg/day TAD + 2.5 mg/kg DOX-treated rat kidney tissue showing moderate distal convoluted tubular congestion (indicated by red thick arrow) with marked lymphocytic infiltration (indicated by blue thick arrow) (x100 magnification, Hematoxylin & Eosin stain) (2 f); (vii). 5 mg/kg/day TAD + 2.5 mg/kg DOX-treated kidney tissue showing focal glomerular atrophy with widening of Bowman's capsule (indicated by black thick arrow) (x100 magnification, Hematoxylin & Eosin stains) (2 g); (viii). 10 mg/kg/day TAD + 2.5 mg/kg DOX-treated kidney tissue showing diffuse tubuloglomerular hypercellularity (indicated by blue thin arrow) and normal tubules (x100 magnification, Hematoxylin & Eosin stains) (2 h). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

study observed a significant elevations in the serum AST at 2.5 mg/kg/day, 5 mg/kg/day, and 10 mg/kg/day TAD pretreatments in the DOX intoxicated rats which is in tandem with the findings of previous studies [56,57] while TAD significantly lowered the serum ALT and ALP levels.

Other notable findings of this study are the effects of DOX intoxication on the antioxidant defense system of the treated rats which was marked by decrease in glutathione (GSH) level, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase and catalase (CAT) activities [52,58,59]. The findings of this study were similar to those earlier reported as the current study is evidenced by the significant decreases in the hepatic SOD, CAT, GST, and GPx activities, and hepatic GSH levels as well as a profound increase in the hepatic tissue MDA activities. However, oral SIL and graded TAD pretreatments reversed these alterations suggesting that TAD offers significant antioxidant effects by restoring their activities to normal levels. Although, hepatic tissue CAT activities that were profoundly decreased by TAD-only pretreatment in our study which is at variance with findings on the effect of TAD on tissue CAT activities, literature indicates that on rare occasions, CAT activities could be depressed by TAD [60].

The kidneys are intricate organs that are essential to keeping healthy bodily functions that are necessary for human survival [61,62]. The kidney is involved in the body's detoxification processes, protein synthesis, especially for blood formation, fluids and electrolytes balance, and excretion or retention of different substances by individual physiological requirements [63]. Numerous chemotherapeutic drugs cause nephrotoxicities that can be detrimental to the patient's health, one of which is DOX which is notorious for causing off-target nephrotoxicity despite its efficacy as an anticancer drug [39,64].

On renal function parameters, the fact that there was a profound increase in the serum renal function parameters as indicated by the significant increase in the serum potassium (K^{+}), urea, and creatinine levels and significant decrease in serum total calcium (TCa^{2+}), bicarbonate (HCO_3^{-}), and chloride (Cl^{-}) levels in rats treated with DOX-only suggested that DOX-induced nephrotoxicity was fully established. Studies have shown that DOX-induced nephrotoxicity is marked by derangements in the renal function parameters which include elevation in blood urea, creatinine, uric acid, and blood urea nitrogen [64–67]. In this study, repeated DOX-only treatment led to a decrease in the kidney's ability to sufficiently excrete urea, potassium, and creatinine, which increased their serum levels. Similarly, reduction in renal functions as recorded in this study hindered the re-absorption of calcium, bicarbonate, and chloride, which resulted in the reduction of their serum levels. However, daily oral SIL and TAD pre-treatments reversed the alterations in the serum levels of these renal function parameters, indicating the renoprotective potential of TAD against DOX-induced renal toxicity.

Oxidative stress is considered a major biochemical highlight of DOX-induced nephrotoxicity [64,67,68]. Oxidative stress results from an imbalance between the reactive oxygen species (ROS) generation and release and endogenous antioxidant activities at neutralizing their toxic effects [65]. DOX intoxication resulted in significant alterations in the renal tissue antioxidant activities that were marked by a profound decrease in the renal superoxidase dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and glutathione peroxidase (GPX) activities, and a significant decrease in renal glutathione levels. These recorded alterations in the renal antioxidant status are in complete agreement with other findings [64,68,69]. Tissue antioxidant enzymes such as SOD, CAT, GST, and GPx fight and mop up free radicals that

could be injurious to body tissues and result in diseases. A decrease in their activities in the renal tissues exposes the kidney to oxidation, resulting in oxidative stress renal injury, and attendant renal failure [70]. Similarly, DOX intoxication is marked by a significant increase in renal tissue malondialdehyde (MDA) activities, the overproduction of which forms the etiopathological basis of lipoperoxidation in renal injury [70,71]. However, daily oral SIL, and TAD pre-treatments significantly reversed the DOX-induced alterations in the renal tissue antioxidant status and restored their activities to normal values. This finding suggested profound antioxidant activity intrinsic in TAD and confirmed earlier reports of this activity [19]. Thus, this corroborated the fact that TAD could be mediating its renoprotective effect via antioxidant and/or free radical scavenging mechanism(s).

The effect of DOX on the hepatorenal pro-inflammatory markers such as IL-1β, IL-6, and TNF-α was also profound. In this study, DOX intoxication was associated with marked increases in the hepatorenal levels of these pro-inflammatory cytokines and corroborating the establishment of hepatorenal toxicity in the treated rats following the repeated DOX administration for 12 days. Inflammation is well documented in the literature as one of the cytotoxic and off-target toxicity mechanisms of DOX [72–74]. Inflammation is generally associated with the increased tissue expressions of IL-1β, IL-6, and TNF-α which are considered reliable markers of inflammation [75–78]. The fact the hepatorenal tissue levels of these pro-inflammatory cytokines were markedly reduced by TAD pretreatment showed the anti-inflammatory potential of this drug in DOX-mediated hepatorenal toxicity.

Furthermore, increases in these serum hepatorenal biomarkers following DOX administration corresponded with remarkable histopathological lesions as revealed by the hepatic and renal tubule degeneration, infiltration of inflammatory cells, as well as histological derangement in the hepatorenal tissues, thus, corroborating those of other studies [79–83]. These results showed that TAD significantly improved the levels of these serum and tissue hepatorenal biomarkers as well as ameliorated DOX-induced hepatorenal tissue histopathological damage.

Overall, the study highlight the potential therapeutic benefit of TAD as an effective adjuvant in preventing the off-target DOX-induced hepatorenal toxicity which may be mediated via antioxidant and anti-inflammatory mechanisms.

Ethics approval

Ethical approval (with the reference number: LASU/23/REC/083) for the study was obtained from the Lagos State University Research Ethics Committee, Lagos State University, Ojo, Lagos State, Nigeria.

Author statement

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Adejuwon Adewale Adeneye: Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Data curation, Conceptualization. **Ademilayo Eunice Adesiji-Adelekan:** Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis. **Fidaraoluwa Esther Babatope:** Writing – original draft, Methodology, Investigation, Funding acquisition. **Ikechukwu Innocent OKOYE:** Resources, Investigation, Formal analysis, Data curation. **Olufunke Esan Olorundare:** Writing – review & editing, Validation, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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