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Low-dose quercetin at 25 mg/kg ameliorates dolutegravir-lamivudine-tenofovirdisoproxilfumarate-inducedcardio-hepato-renal toxicities in Wistar rats

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Abstract

Combination antiretroviral therapies (cARTs) are linked with multiple-organ system (MOS) toxicities in laboratory animals, and in humans undertaking treatment for HIV/AIDS. The ameliorative potential of low-dose quercetin following cART-associated MOS-toxicities in cardio-hepato-renal organs was evaluated in in vivo model. Oral administration of cART (Dolutegravir 50 mg, Lamivudine 300 mg and Tenofovir disoproxil fumarate 300 mg [DLT]) at 9.29 mg/kg, was challenged against low-dose quercetin 25 mg/kg body weight (bw) in Wistar rats. Group 1, the normal control (NC) received distilled water (5 mL), while groups 2 to 4 received quercetin (25 mg), DLT (9.29 mg), and DLT + quercetin (9.29 mg + 25 mg respectively), per kg bw. All administrations lasted for 14 days, and thereafter animals were humanely sacrificed after intraperitoneal anesthesia injection with 100 mg ketamine /5 mg xylazine per kg bw followed by cervical dislocation. Blood and organs were harvested for analyses using standard protocols. The serum concentrations of lipid parameters [total cholesterol, triglycerides, LDL-cholesterol, and VLDL-cholesterol], liver biomarkers (total-bilirubin, direct-bilirubin, and transaminases], and kidney biomarkers [urea and creatinine] were significantly increased ($p < 0.05$) while electrolytes (Na^+, K^+, Cl^- and HCO_3^-) were significantly decreased ($p < 0.05$) in DLT group but improved in DLT + Q group. Histopathology demonstrated distorted myocytes, hepatocytes and renal tubules, fatty liver with vacuolization, dystrophied glomeruli and distorted renal interstitium in DLT group, compared with normal appearing histoarchitectural features in NC and DLT + Q groups. In conclusion, oral administration of low-dose quercetin (25 mg/kg) ameliorated cART-associated cardio-hepato-renal toxicities in rats, improving their biomarkers and histoarchitecture.

Keywords Combination antiretroviral therapies, Multiple-organ toxicities, Quercetin, Liver and kidney functions, Lipid profile

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Background

Combination antiretroviral therapy (cART) involves the concurrent use of a combination of three or more antiretroviral drugs to suppress human immunodeficiency virus (HIV) replication and has resulted in a significant reduction in acquired immunodeficiency virus (AIDS) -related morbidity and mortality, reducing HIV to a manageable disease. Globally, an estimated 39 million are people living with HIV/AIDS (PLWHAs), with some 630,000 reported mortalities resulting from HIV-related causes in 2022 [1, 2]. Africa region has the highest HIV prevalence worldwide with 4.0% in Kenya, 1.3% in Nigeria, 18.3% in South Africa, 18% in Botswana, and 27.9% in Eswatini for individuals aged 15–49 years. Generally, the region accounts for 50% of global new HIV infections [3].

The fixed-dose combination of dolutegravir, lamivudine and tenofovir disoproxil fumarate had been approved in Germany in 2018 for the treatment of HIV-1 in adults [2]. However, dolutegravir has been reported to induce hepatotoxicity by elevating liver enzymes [4]. Lamivudine is associated with an increase in liver enzyme activities and structural damage to liver cells [5], in addition to hyperlipidemia and declining renal function [6]. Tenofovir disoproxil fumarate had been reported to induce nephrotoxicity by decreasing glomerular filtration rate, elevating liver enzymes [7], and playing a role in pathogenesis of cardiovascular diseases [8]. Consequently, these reports of their adverse effects resulting in organotoxicities, gross neurological and cognitive dysfunction in experimental animals have become a growing concern [9, 10]. Dolutegravir (DTG), lamivudine (3TC) and tenofovir disoproxil fumarate (TDF) in the long-term have also been associated with the onset of metabolic complications [9, 11].

Quercetin is a potent antioxidant that belongs to the flavonoid group and is generally present as Que glycoside. It can be sourced from apples, berries, black tea, cherries, citrus fruits, grapes, onions, buckwheat, kale, red wine, and tomatoes [12]. The required human dietary intake of all flavonoids is estimated between 200 and 350 mg/day, while the normal daily ingestion of flavanols varies from 20 to 35 mg, of which quercetin accounts for nearly 50%, with a daily consumption of about 10 mg/day [12, 13].

A broad spectrum of pharmacological properties has been described for quercetin, including neuroprotective, anticancer, cardioprotective, antioxidant, antiviral, antimicrobial, antithrombotic, antiapoptotic, anti-inflammatory and hepatoprotective [13–15]. Quercetin has been reported to enhance antioxidant capacity and regulates autophagy loss by reducing the stabilization of β-catenin and hypoxia-inducible factor 1-alpha (HIF-1α) and inhibiting the phosphorylation of Ak strain transforming (AKT), mammalian target of rapamycin (mTOR), and extracellular signal-regulated kinase (ERK) [16]. However, quercetin at high doses has been observed in a previous (unpublished) study to elicit multiple organ toxicities (involving the heart, liver and kidney among others) in Wistar rats.

The heart delivers oxygen- and nutrient-rich blood to tissues and organs, and cART administration has been reported to induce atherogenesis, endothelial dysfunction, and coagulation abnormalities, probably through inflammation and immune dysregulation [17, 18]. Lipid profile assessment plays an important role in the pathogenesis and management of various cardiovascular diseases [19].

The liver is a vital organ in the body with varieties of functions ranging from drug detoxification to bile production [17, 20]. Liver function tests are utilized in the determination and assessment of liver damage. Parameters normally assayed include serum activities of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in addition to serum concentrations of bilirubin and albumin [21].

The kidney participates in the control of the volume of various body fluids, fluid osmolality, acid-base balance, various electrolyte concentrations and removal of toxins. Likewise, fluid filtration occurs in the glomerulus [17, 22]. Serum creatinine and urea concentrations play an important role in the diagnosis and management of different kidney injuries and also serve as a measure of glomerular filtration rate utilized in kidney impairment [23].

Translational studies permit allometric scaling wherein scientists exchange doses between species during research for possible prediction of animal study to human condition [24, 25]. It is estimated that quercetin be consumed at a dose ranging from 25 to 50 mg/day among people with equilibrated nutrition [26]. In view of the possibility of quercetin to elicit organ toxicity at high doses, a short duration study using a low dose was considered. This sub-chronic (14-day) study was aimed at evaluating the ameliorative potential of low-dose quercetin at 25 mg/kg on cardio-hepato-renal functions and organ microanatomies following dolutegravir-lamivudine-tenofovir disoproxil fumarate oral administration to Wistar rats.

Materials and methods

Experimental animals

The experimental animals (young male and female Wistar rats; 117 to 181 g) were sourced from the Animal House of the Faculty of Pharmacy, Department of Pharmacology and Toxicology, University of Uyo, Nigeria. The protocols for this study were approved by the Department of Human Anatomy, University of Uyo, Nigeria, which aligns with the globally accepted guideline [27], with ethical approval obtained from the Health Research Ethics Committee of the Akwa Ibom State Ministry of Health

(Ref: MH/PRS/99/Vol.IV/697). The study was conducted in accordance with the Basic and Clinical Pharmacology and Toxicology policy for experimental studies [28]. All animals were weighed, marked for identification, and placed in a standard plastic cage for acclimatization for one week under optimum pathogen-free environment and maintained in a 12 h light/dark cycles of 25–27ºC at relative humidity of 40–60% measured using the CEM hydrometer (DT 615, Shenzhen China). All animals were fed with pelletized growers mash (Grand Cereal Vital® Feed Ltd. Jos) and provided with drinking water *ad libitum*. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines 2.0 as checklist of relevant information for animal research reporting of in vivo experiments was consulted [29].

Drug acquisition and preparations

The drugs used in this study were quercetin (500 mg) and cART (dolutegravir 50 mg+lamivudine 300 mg+tenofovir disoproxil fumarate 300 mg). Quercetin was obtained from aSquared Nutrition Brand LLC Miami, USA with Lot No. X001EWXNWV.The cART was obtained from Mylan Laboratories Limited, India (with NAFDAC Registration No: 134–9927 and Batch No.3124536). The drugs (normally in solid form) were pulverized in a mortar and pestle and diluted in 100 mL of distilled water to constitute stock solution.

The drugs were administered orally using oro-gavage intubation with the determination from the animal's snout tip to its last rib. Thus, when put at a distance, the tube's tip was in the stomach or very distal esophagus.

Experimental design

Group	Treatment	Treatment Duration (days)
NC	Distilled water (5 mL/kg body weight (bw)	14
Q	Quercetin 25 mg/kg (bw)	14
DLT	DLT 9.29 mg/kg bw	14
DLT+Q	DLT (9.29 mg/kg bw)+Quercetin (25 mg/kg bw)	14

Legend

NC=Normal control, Q=Quercetin; DLT=Dolutegravir (50 mg)+Lamivudine (300 mg)+Tenofovir disoproxil fumarate (300 mg). DLT+Q=DLT+Quercetin.

Termination of experiment and sample collection

Animals were humanely sacrificed 24 h after their last administration, under anesthesia via 100 mg ketamine /5 mg xylazine per kg bw intraperitoneal injection followed by cervical dislocation. Blood samples were collected via cardiac puncture method into centrifuge tubes which were left to clot at room temperature for 45 min and then centrifuged at 3000 rpm for 15 min. The clear non-hemolyzed supernatant sera were quickly removed and kept at 30 °C for various biochemical analyses. The cardio-hepato-renal organs (hearts, livers, and kidneys) were dissected out, weighed, and stored in 10% neutral buffered formalin for tissue processing and light microscopy.

Determination of organo-somatic indices

The final body and organ weights (heart, liver, and kidney) were recorded. Organo-somatic index was calculated using the formula: organ weight (g)/ final body weight (g) x 100.

Assay of lipid profile

Lipid profile test assay was done using assay chemistry kits from Agappe Diagnostics, (Switzerland GmbH; Lot numbers: TG – 33030320; CHOL – 33030211 and HDL – 32120037), based on the methods of Allain [30], Hafiane and Genest [31], Bucolo and David [32] and Contois et al., [33]. Estimation of VLDL cholesterol was done by calculations based on Friedewald et al., [34].

Assay for liver function tests

Liver function assays were done using chemistry kits from Agappe Diagnostics, Switzerland GmbH (Lot numbers: SGOT-32120327 and SGPT −32120236). Alanine amino transaminase (ALT) and aspartate amino transaminase (AST) were assayed by kits based on the method of Reitman and Frankel [35]. Alkaline phosphatase (ALP) was estimated as described by Schlebusch et al., [36].

Determination of serum bilirubin

Concentrations of total and direct bilirubin in serum were determined utilizing kits from Agappe Diagnostics, Switzerland GmbH (Lot number: Direct & Total Bilirubin 32110457), based on the method described by Walter and Gerard [37].

Determination of serum concentrations of sodium, potassium, chloride, and bicarbonate

Sodium, potassium, and chloride determinations were done with kits from Agappe Diagnostics, Switzerland GmbH (Lot numbers: Na – 32150311; K – 32160177; Cl – 32170215). Serum bicarbonate was assayed using a kit from Teco Diagnostics, California, USA (Lot number: HCO3–83725) following the method described in Tietz by [38].

Determination of urea and creatinine concentrations

Urea and creatinine concentrations were determined using kits from Agappe Diagnostics, Switzerland GmbH (Lot number: Urea– 33030386 and Creatinine −32160144), based on the methods described by Weatherburn [39] and Haeckel [40] respectively.

Table 1 Effect of quercetin on body weight after administration of DLT

Group	Body Weight (g)			
	Initial	Final	Weight Change	%Weight Change
NC	143.0 ± 4.22	149.0 ± 5.0	6.00	4.02
Q	127.5 ± 5.24	134.0 ± 5.40	6.50	4.85
DLT	155.5 ± 6.18	127.0 ± 10.4	-28.50[a]	-22.44 [a]
DLT + Q	151.75 ± 13.40	163. 0 ± 13.9	11.25[a, b]	6.90 [a, b]

Values are mean ± SEM, (n = 5 rats/group)

a = p < 0.05 relative to NC and Q; b = p < 0.05 relative to DLT.

Legend

NC = Normal control; Q = Quercetin (25 mg/kg body weight [bw]); DLT = Rats given DLT (Dolutegravir [50 mg] + lamivudine [300 mg] + tenofovir disoproxil fumarate [300 mg]) at 9.29 mg/kg bw; DLT + Q = Rats administered DLT (9.29 mg/kg bw) + quercetin (25 mg/kg bw).

Histopathological assessments

Haematoxylin and Eosin (H&E) stain

The histological alterations were assessed from formalin fixed paraffin embedded blocks of cardio-hepato-renal tissues from the study. The 5 µm serial sectioned tissues picked on albuminized slides (every 5th ribbon sections) were deparaffinized using 2 changes of xylene for 5 min each, and thereafter through rehydration in descending grades of alcohol, absolute, 95% and lastly 70% for 5 min each. The tissues were then transferred to running tap water for 15 min to remove any infiltrating agent. Thereafter tissue sections were immersed in haematoxylin for about 30 s, and then rinsed in running tap water, then transferred to eosin stain for about 1 min. The tissue sections were rinsed again before dehydrating in ascending grades of alcohol 50%, 60%, 70%, 80%, 95%, and 100%, respectively. The tissue sections were cleared in xylene, mounted using DPX (dibutylphthalate polystyrene xylene), cover-slipped and blot-dried [41]. A light microscope (Olympus - CX31, Japan) with an attached AmScope® digital camera (MU 1000, China) was used to capture the photomicrographs which were blindly assessed by at least 3 independent histopathologists.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 25 was used to analyze the data obtained from this study, which were expressed as means ± SEM. Comparisons of means were determined with a one-way analysis of variance (ANOVA) while a post-hoc test used Tukey for multiple comparison. Test values were considered significant at $p < 0.05$.

Results

Effect of quercetin on body and organ weights after administration of DLT

There were significant ($p < 0.05$) body weight changes in the DLT and DLT + Q groups when compared to NC and Q groups (Tables 1 and 2), respectively.

Effect of quercetin on lipid profile after administration of DLT

The DLT-administered group showed a significant ($p < 0.05$) increase in the serum concentrations of total cholesterol (TC), LDL-cholesterol, VLDL-cholesterol, and triglycerides (TG) when compared to NC and Q groups. The DLT + Q group indicated a return to NC range in the lipid concentrations evaluated (Table 3).

Effect of quercetin on liver function markers after administration of DLT

There were significant ($p < 0.05$) increases in serum concentrations of bilirubin (both total and direct), as were in the serum activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) of the DLT-administered group when compared with the Q and NC groups. The DLT + Q group demonstrated a marked decrease in the elevated liver function makers when compared to the DLT alone group (Table 4).

Effect of quercetin on kidney function markers after administration of DLT

Urea and creatinine concentrations in the DLT-administered group were significantly ($p < 0.05$) increased when

Table 2 Effect of quercetin on organ weights after administration of DLT

Group	Organ Weight (g)			Organosomatic Index (OI)		
	Heart	Liver	Kidney	Heart	Liver	Kidney
NC	0.66 ± 0.03	3.86 ± 0.13	1.04 ± 0.02	0.45 ± 0.03	2.89 ± 0.09	0.70 ± 0.05
Q	0.68 ± 0.02	4.43 ± 0.07	1.05 ± 0.02	0.51 ± 0.02	3.18 ± 0.08	0.78 ± 0.04
DLT	0.71 ± 0.05	4.86 ± 0.29[a]	1.17 ± 0.05	0.57 ± 0.03	3.58 ± 0.21	0.93 ± 0/06[b]
DLT + Q	0.74 ± 0.02	4.73 ± 0.20	1.10 ± 0.04	0.47 ± 0.05	3.08 ± 0.26	0.69 ± 0.08
P value	0.326	0.009	0.061	0.107	0.082	0.0001

Values are mean ± SEM, (n = 5 rats/group), a = p < 0.05 relative to NC; b = p < 0.05 relative to NC, Q and DLT + Q.

Legend

NC = Normal control; Q = Group of rats administered quercetin (25 mg/kg body weight [bw]); DLT = Group of rats given DLT (Dolutegravir [50 mg] + lamivudine [300 mg] + tenofovir disoproxil fumarate [300 mg]) at 9.29 mg/kg bw; DLT + Q = Group of rats administered DLT (9.29 mg/kg bw) + quercetin (25 mg/kg bw).

Table 3 Effect of quercetin on lipid profile after administration of DLT

Group	Parameter (mg/dL)				
	Total Cholesterol	Triglycerides	HDL-Cholesterol	LDL-Cholesterol	VLDL-Cholesterol
NC	130.49 ± 2.41^{cd}	129.22 ± 2.03	30.04 ± 1.15	25.84 ± 0.41	74.59 ± 2.55
Q	128.52 ± 1.17^{cd}	128.98 ± 0.85	29.81 ± 2.42	25.80 ± 0.17	72.91 ± 3.61^{b}
DLT	149.53 ± 2.94^{ab}	134.07 ± 1.79	31.51 ± 0.60	26.81 ± 0.36	91.20 ± 2.16^{a}
DLT + Q	138.80 ± 0.53^{ab}	132.25 ± 2.65	31.06 ± 1.68	26.45 ± 0.53	81.29 ± 2.06 ^{a}
P value	0.0001	0.226	0.856	0.227	0.001

Values are mean ± SEM, (n = 5 rats/group), a = p < 0.05 relative to NC, Q and DLT + Q, b = p < 0.05 relative to DLT + Q; ab = p < 0.05 relative to NC and Q, cd = p < 0.05 relative to DLT + Q.

Legend

HDL = High density lipoprotein, LDL = Low density lipoprotein, VLDL = Very low-density lipoprotein; NC = Normal control; Q = Group of rats administered quercetin (25 mg/kg body weight [bw]); DLT = Group of rats given DLT (Dolutegravir [50 mg] + lamivudine [300 mg] + tenofovir disoproxil fumarate [300 mg]) at 9.29 mg/kg bw; DLT + Q = Group of rats administered DLT (9.29 mg/kg bw) + quercetin (25 mg/kg bw).

Table 4 Effect of quercetin on liver function markers after administration of DLT

Group	Parameter				
	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Aspartate Transaminase (U/L)	Alanine Transaminase (U/L)	Alkaline Phosphatase (U/L)
NC	0.60 ± 0.10	0.39 ± 0.05	129.00 ± 1.26	58.66 ± 2.74	21.44 ± 1.32
Q	0.62 ± 0.54	0.45 ± 0.07	135.45 ± 2.40^{cd}	63.20 ± 2.50^{b}	29.80 ± 1.13^{ab}
DLT	0.92 ± 0.02^{a}	0.52 ± 0.01	141.74 ± 2.50^{a}	79.65 ± 1.55^{a}	30.46 ± 0.79^{ab}
DLT + Q	0.68 ± 0.23^{b}	0.38 ± 0.04	140.63 ± 3.15^{a}	77.59 ± 3.44^{a}	23.08 ± 2.27^{bd}
P value	0.005	0.191	0.007	0.0001	0.001

Values are mean ± SEM, (n = 5 rats/group), a = p < 0.05 relative to NC, b = p < 0.05 relative to DLT, c = p < 0.05 relative to DLT + Q; ab = p < 0.05 relative to NC and DLT + Q, bd = p < 0.05 relative to Q, cd = p < 0.05 relative to DLT.

Legend

NC = Normal control; Q = Group of rats administered quercetin (25 mg/kg body weight [bw]); DLT = Group of rats given DLT (Dolutegravir [50 mg] + lamivudine [300 mg] + tenofovir disoproxil fumarate [300 mg]) at 9.29 mg/kg bw; DLT + Q = Group of rats administered DLT (9.29 mg/kg bw) + quercetin (25 mg/kg bw).

Table 5 Effect of quercetin on kidney function markers after administration of DLT

Group	Parameter					
	Electrolyte				Urea (mg/L)	Creatinine (μmol/L)
	Chloride Cl^- (mEq/L)	Bicarbonate HCO_3^- (mmol/L)	Potassium K^+ (mmol/L)	Sodium Na^+ (mmol/L)		
NC	111.29 ± 1.45	18.15 ± 0.56	3.85 ± 0.16	139.07 ± 2.35	12.59 ± 0.26	0.68 ± 0.11
Q	105.55 ± 2.57	18.12 ± 0.34	3.66 ± 0.42	135.55 ± 2.83	13.69 ± 0.53	0.74 ± 0.11
DLT	103.59 ± 0.58	18.24 ± 0.14	3.27 ± 0.27	121.97 ± 4.32^{ab}	15.08 ± 0.36 ^{a}	0.94 ± 0.03
DLT + Q	105.71 ± 4.16	18.14 ± 0.33	3.42 ± 0.16	132.10 ± 2.35	14.54 ± 0.83	0.89 ± 0.04
P value	0.214	0.995	0.475	0.007	0.005	0.110

∗Values are exposed as mean ± SEM. (n = 5); a = p ˂ 0.05 relative to NC; ab = p ˂ 0.05 relative to N, Q, DLT + Q.

Legend

NC = Normal control; Q = Group of rats administered quercetin (25 mg/kg body weight [bw]); DLT = Group of rats given DLT (Dolutegravir [50 mg] + lamivudine [300 mg] + tenofovir disoproxil fumarate [300 mg]) at 9.29 mg/kg bw; DLT + Q = Group of rats administered DLT (9.29 mg/kg bw) + quercetin (25 mg/kg bw).

compared with NC and Q groups. However, the DLT + Q group had significant (p < 0. 05) decreased urea and creatinine concentrations when compared to DLT-group. The concentration of serum electrolytes Na^+, K^+, Cl^-, and HCO_3^-significantly (p < 0. 05) decreased in the DLT administered group compared to the NC and Q groups (Table 5).

Effect of quercetin on cardio-hepato-renal histopathological alterations after administration of DLT

The photograph of the heart transverse section of DLT-administered group showed mildly distorted and hypertrophied myocytes with few vacuolations. The DLT + Q-administered group appeared normal and like NC and Q groups with centrally arranged nucleus, in addition to normal appearing myofibers and connective tissues (Fig. 1).

The liver of the DLT-administered group demonstrated diffuse and prominently degenerated and atrophied

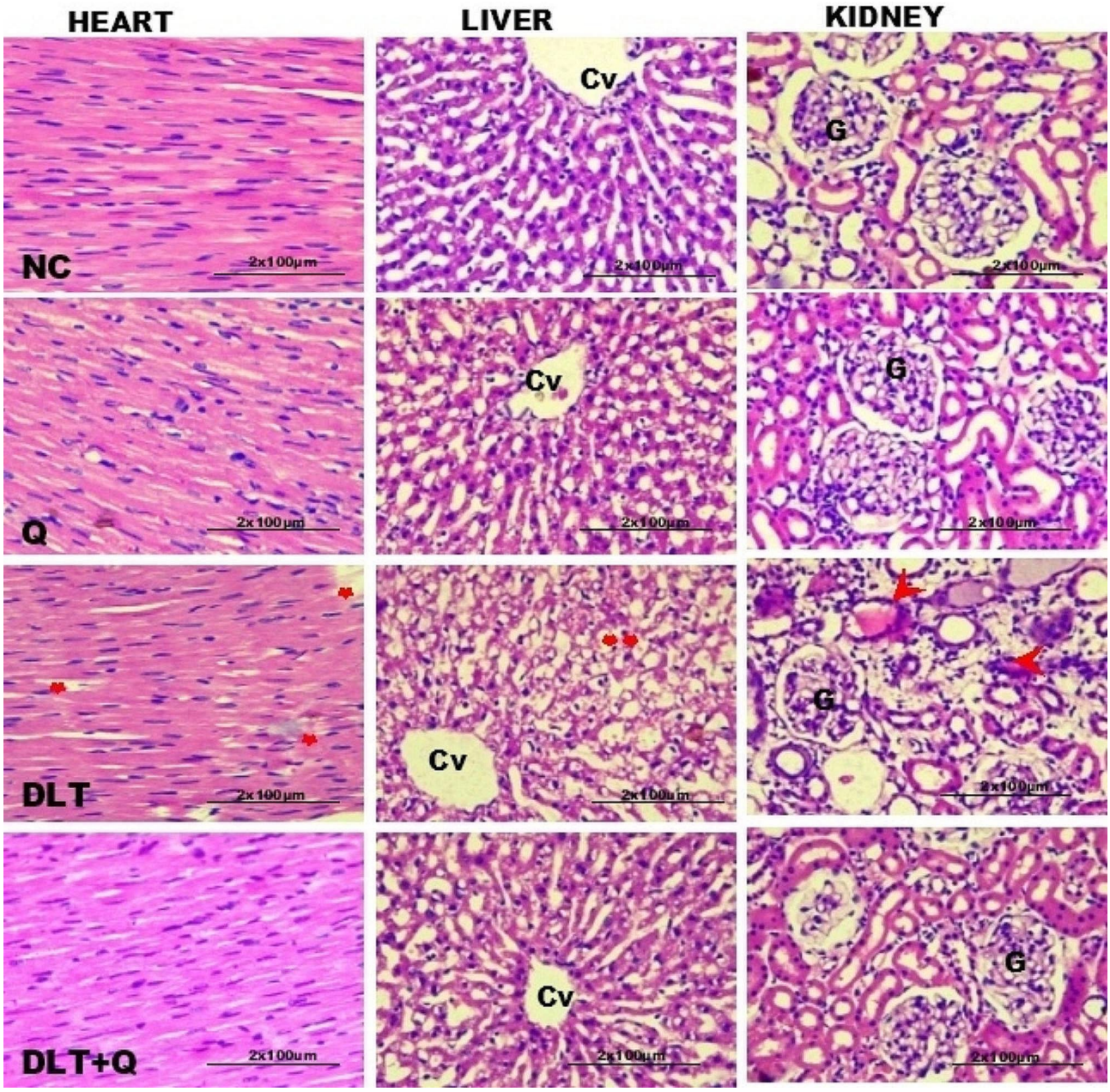

Fig. 1 Photomicrographs of cardio-hepato-renal tissues of experimental rats following administration of quercetin against dolutegravir-lamivudine-tenofovir disoproxil fumarate-induced organ toxicity (H&E: x400)

Red star = distorted myocytes; Double asterisks = prominent hepatic steatosis and vacuolization; red arrowhead = degenerating tubules and interstitium.

Legend

NC = Normal control; Q = Rat administered quercetin (25 mg/kg body weight [bw])

DLT = Rat given DLT (Dolutegravir [50 mg] + lamivudine [300 mg] + tenofovir disoproxil fumarate [300 mg]) at 9.29 mg/kg bw. DLT + Q = Rat administered DLT (9.29 mg/kg bw) + quercetin (25 mg/kg bw).

hepatocytes and sinusoids, with profuse vacuolations and steatosis. The DLT + Q administered group presented a recovery trend in the arrangement of the arrays of hepatic plates, sinusoids from the central vein, with no diffuse vacuolations and steatosis, similar to the normal appearing histoarchitecture in the NC and Q groups when compared with the DLT-administered group (Fig. 1).

The transverse section of the kidney indicated widespread degeneration in the renal parenchyma, affecting the renal tubules and interstitium with multiple atrophic

glomeruli, altered Bowman's/ urinary space and focal inflammatory cells in the DLT-administered group. The DLT+Q- administered group demonstrated more improved histoarchitecture than the DLT group, with fewer atrophic glomeruli and distorted renal tubules, and had an appearance like Q and NC groups (Fig. 1).

Discussion

In the management of HIV/AIDS (a major chronic health global challenge, especially in the African continent), combination antiretroviral therapy (cART) is the approved and most effective therapy [1–3]. DLT (dolutegravir+lamivudine+tenofovir disoproxil fumarate) is a currently popular cART administered to PLWHAs in Africa. Quercetin on the other hand, is a powerful antioxidant that possesses cardioprotective, antioxidant, anti-inflammatory and hepatoprotective properties [13–15].

Estimating the changes in markers and biochemical properties is pertinent in understanding and identifying the pathogenesis induced by toxins and/or therapies to the body in addition to improving clinical outcomes [42, 43]. Previous studies have reported the toxicity of cART administration [10, 11]. In this study, we investigated the ameliorative potential of quercetin at 25 mg/kg against therapeutic dose of DLT-induced cardio-hepato-renal toxicities in rats.

This study revealed an increase in body weight in all groups except DLT+Q group. The decrease in DLT+Q group may be due to protein wasting or morphological changes [44]. A significant increase in organ weight in DLT group may suggest an onset of a disorder which is associated with organ hypertrophy [44]. Identification of potential harm by a drug can be deduced from organ weight in toxicological examination [45].

Dyslipidemia (one of the major causes of heart diseases) is associated with the accumulation of lipids in the heart and reveals onset of cardiovascular disease [19]. There could be increases in levels of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) leading to their accumulation in layers of blood vessels. These affect blood vessel constriction in the heart, causing subsequent induction of arteriosclerosis and thrombocytopoiesis [19]. There were mild distortions of myocytes and hypertrophy in the DLT-administered group when compared to the NC group. Although cardiac myocyte proliferation and regeneration is rare in humans, the regulation of cellular stress, immune system and deposit of extracellular matrix limit harmful nature of the cardiac injury and help cardiac myocytes to heal from insults [20].

People living with HIV/AIDS are susceptible to coronary heart disease (CHD), which is one of the cardiovascular diseases (CVDs) that are now presently causing death and different illnesses among HIV patients [46]. The present study showed significant increases in concentrations of TG, TC, LDL-C and VLDL-C in the DLT group when compared with the Q, DLT+Q and NC groups.

The increased concentrations of TG, TC, LDL-C and VLDL-C in the QLT-administered group may be associated with lipid metabolism changes [43]. LDL-C serves as the principal lipid target to reduce cardiovascular risk in the management of dyslipidemias, and its increase indicates the onset/likelihood of CVDs [47, 48]. Nucleoside-reverse transcriptase inhibitors (NRTIs), a class of antiretroviral therapy, have been reported to induce changes in lipid metabolism [49]. The lamivudine and tenofovir disoproxil fumarate in the DLT used in this study demonstrated dyslipidemia activity. NRTIs reportedly cause the accumulation of lipid within adipocytes, leading to mitochondrial dysfunction and depletion of mitochondrial DNA (mtDNA). They also down-regulate the expression of peroxisome proliferator-activated receptor gamma (PPARγ) in adipose tissue, leading to lipid metabolism changes and dyslipidemia [50], as demonstrated in the group administered DLT when compared with the Q and NC groups. It is pertinent to note that co-administration of DLT+Q caused decreases in concentrations of TC, VLDL-C, TG, and LDL-C when compared with administration of DLT-alone, suggesting a potential of quercetin to ameliorate dyslipidemia associated with DLT administration. Previous study has reported that quercetin improves lipid profile in rats and exerts cardioprotective effects [51], which is in consonance with our observations on DLT+Q group when compared with DLT and NC groups.

Elevation of liver function test parameters above normal serum concentrations can lead to the confirmation of various hepatocellular diseases [21]. Hepatic progenitor cells play an important role in liver regeneration through differentiation in hepatocytes [52]. The liver is a site for metabolism, detoxification, and the synthesis of different enzymes [42]. Elevation of liver enzymes is common in patients taking HAART, which may be through its active metabolites or by direct toxicity [42]. The severity of liver cell injury can be determined by measuring the changes in concentration of transaminase enzymes, which are significantly increased during insult or toxicity in the liver [42]. In the diagnosis of various acute hepatocellular injuries, the concentrations of ALP, ALT and AST serve as the most sensitive indicators [42, 53].

In this study there were significant increases in bilirubin concentrations and activities of ALP, ALT, and AST in the DLT group when compared with Q, DLT+Q and NC groups, demonstrating hepatotoxic effects of DLT. ALP is secreted by the bone and other tissues. A significant increase in ALP activity, as shown in the DLT group compared to other groups, can be associated with

increased production of isoenzymes of liver ALP induced by HAART administration. Several studies [42, 53] have reported that AST and ALT produced by the liver, kidney, heart, and skeletal muscles are deposited in the plasma in great amounts when there is an insult to the liver cell membrane resulting in increased permeability, which supports our findings that DLT elicited increases in serum activities of ALT and AST, suggesting hepatotoxic potentials. It is also worthy of note that increases in total and direct bilirubin concentrations suggest deficiencies in bilirubin processing because of DLT administration.

Quercetin is reported to possess iron-chelating ability, which can help ameliorate iron overload and protect the liver against oxidative stress, protein oxidation and lipid peroxidation [54]. The DLT+Q administered group in this study showed a decrease in bilirubin concentrations and activities of ALP, ALT and AST when compared to the DLT group. In this study the co-administration of DLT+Q seemed to attenuate the surge in liver enzymes (AST, ALT, and ALP) and bilirubin concentrations caused by DLT administration. This finding confirms that quercetin has hepatoprotective potential and helps in ameliorating hepatic tissue damage while also protecting the structural integrity of the hepatocellular membrane, subsequently preventing enzyme leakage into the bloodstream [55]. This is evident in the decreased activities of transaminases (ALT and AST) and ALP in DLT+Q group, which became almost similar to that of the NC and Q groups. Also, moderate to severe alterations were demonstrated in the histoarchitectural pattern of the liver in the DLT-administered group when compared to Q and NC groups. Changes observed included hepatic steatosis, vacuolations and abundant red blood cells in the sinusoid. These findings suggested drug (DLT)-induced changes [45, 56], which were supported by biochemical parameters in this study.

The kidney is an organ with excretory and regulatory functions in the body. Abnormal function of the kidney can result from infections, cancer, and toxic chemicals [57]. A progressive reduction in kidney function can result in chronic kidney disease (CKD) and end-stage renal disease (ESRD) [58]. Electrocyte homeostasis is maintained by the kidney, and any injury to the kidney can lead to disturbance in electrolyte level and the most common electrolyte associated with kidney disorders are sodium, calcium, and potassium [59]. Assessment of serum electrolytes (Na^+, K^+, Cl^-, and HCO_3^-), urea and creatinine are important biochemical markers, can be pivotal in the proper diagnosis of dysfunction associated with the kidney [59]. The body consists of extracellular and intracellular fluid compartments that contain different inorganic electrolytes that aid in water and electrolyte movement between the body compartments to help maintain body homeostasis [57].

With a low regenerative potential, tubular epithelial cells of the kidney possess the ability to proliferate during injury [60].

Urea and creatinine are waste products of metabolism usually excreted by the kidney through glomerular filtration and utilized as good indicators of a normally functioning kidney [61]. The result of this study demonstrated significant increases in urea and creatinine concentrations in the group administered DLT when compared with the NC, Q and DLT+Q groups. This suggests a diminution in glomerular filtration since creatinine and urea are filtered by the glomerulus and are utilized indirectly in measuring glomerular filtration rate (GFR) [58]. A decrease in GFR is associated with an increase in plasma levels of creatinine and urea, resulting in various kidney dysfunctions, including glomerular inflammation and interstitial nephritis [57, 61], as seen in the DLT-administered group. Concentrations of Na^+, K^+, Cl^-, and HCO_3^- significantly decreased in the DLT group compared to the NC, Q and DLT+Q groups, suggesting excessive loss of water from the body fluid.

The results suggest that DLT may have impaired the Na^+/K^+ pump and membrane-bound aldosterone that helped regulate the absorption of sodium into the cell, subsequently resulting in renal dysfunction [57]. Antiretroviral drugs induce renal failure through different mechanisms, one of which includes direct renal tubular toxicity, crystal deposits on the kidney, and glomerular lesions, especially with the NRTIs class. Changes in the kidney are characterized by degenerated and dilated renal tubules with atrophic glomeruli. Decline in glomerular filtration rate is often due to decrease in surface area of glomeruli available for filtration which result to reduction in metabolic activity [44, 45]. It is noteworthy in this study that quercetin at 25 mg/kg improved kidney function and histoarchitecture, which is evident in the decreased urea and creatinine concentrations detected in the DLT+Q administered group when compared with the DLT group. Quercetin mechanisms involve scavenging free radicals, chelating metal ions and inhibiting lipid peroxidation [62] suggesting its nephroprotective potential. In this study, the kidney function test agrees with H & E assessments supporting the link that DLT-induced kidney injury.

Pathological and microstructural alterations demonstrated in the DLT-administered group were ameliorated in DLT+Q group further confirming the ameliorative potential and antioxidant properties of quercetin as in other studies [13, 15, 44]. Co-administration of quercetin and HAART as seen in DLT+Q mitigated cART-induced histopathological alterations. Previous findings [44] correlate with the micro-anatomical assessments of this study.

Conclusion

In conclusion the sub-chronic phase oral administration of low-dose quercetin at 25 mg/kg demonstrated ameliorative potential against dolutegravir-lamivudine-tenofovir disoproxil fumarate-induced cardio-hepato-renal toxicities in Wistar rats possibly through its potent antioxidative pathway by alleviating dysregulations of lipid profile, liver and kidney biomarkers and an improvement in the histoarchitecture of cardio-hepato-renal organs.

Author contributions

Conceptualization: IAE, ANA, DOE. Data acquisition: BUB, RSI, DCE. Data analysis or interpretation: IAE, MAA. Drafting of the manuscript: IAE, DOE. Critical revision of the manuscript: IAE, ANA, MAA, DOE. Approval of the final version of the manuscript: all authors.

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Data availability

All data derived from this study was included in this article.

Declarations

Consent for publication

Not applicable.

Competing interests

Authors declare that there is none.

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